VOLUME III DECEMBER, 1910 PART IV

SOME CHARACTERISTICS OF THE WESTERN PRAIRIE SOILS OF CANADA[1].

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IT will be desirable, by way of preface, to say a word or two regarding the physical geography of the district, the soils of which form the subject of this paper.

Physical Geography of the Great Plains Region in Canada.

The term prairies as applied to the Canadian west is used to denote all that portion of the Great Plains Region north of the 49th parallel found within the confines of the three western provinces, Manitoba, Saskatchewan and Alberta, and northward to the Arctic Ocean. Commencing some fifty miles east of Winnipeg, at the western edge or rim of the Laurentian area, it extends westward to the foothills of the Rockies—though it must not be supposed that this immense stretch of country, some 800 miles if measured near the southern boundaries of these provinces, is one continuous, uninterrupted prairie or plain. It is rather a series of three great plains or plateaux, marked off by more or less distinct lines of escarpment which frequently take the form of ridges and wooded hills. Considering the region as a whole, it is found to narrow as we proceed northward, contracting to about 400 miles at the 56th parallel, and north of the 62nd parallel to still less, but further northward again expanding to its termination on the Arctic Ocean. It may therefore be considered as, in general, a huge wedge-shaped area extending northward and with its base lying along the Canadian frontier.

[1] Read before the Agricultural Subsection of the British Association for the Advancement of Science, Winnipeg, Aug. 27th, 1909.

"The southern part of this great plain," wrote[1] the late Dr Geo. M. Dawson twelve years ago, then Director of the Geological Survey of Canada, "is not only the most important from an economic point of view, but also that about which most is known. It includes the wide prairie country of the Canadian west, with a spread of about 193,000 square miles of open grass land, an area more than twice that of Great Britain. Beyond the North Saskatchewan river, the plain becomes essentially a region of forest with occasional prairie tracts such as those of the Peace River Valley." Settlement began in the south of this prairie country and every year has seen successful grain growing pushed farther and farther to the north.

The first and lowest of the three steppes or prairie levels which constitute this interior plain is that of the Red River Valley with an elevation of about 800 feet above the sea. Its northern portion is occupied by the Winnipeg group of lakes, and to the south of Lake Winnipeg "it comprises some 7000 square miles of prairie land, which to the eye is absolutely flat, although rising uniformly to the east and west of the river. This is the former bed of the glacial 'Lake Agassiz,' the sediments of which constitute the richest wheat lands of Manitoba."

The second or middle prairie, with an average elevation of 1600 feet, extends from the escarpment forming the western boundary of the first prairie to a second fairly well marked and nearly parallel rise known as the Missouri Coteau. The first escarpment referred to comprises the so-called "Pembina Mountain" in the south and continues in a north-westerly direction including the Riding, Duck, Porcupine and Pasquia hills. The approximate area of this plain is given as 105,000 square miles, more than half of which is stated to be open prairie. It is less regular in its surface than the Red River Valley plateau, undulations, low hills and ridges being not uncommon. The soil is by no means as uniform in character as that of the first prairie, though large areas are of exceedingly fine quality and extremely fertile.

The third steppe, with an average elevation of 3000 feet, continues from the Missouri Coteau to the Rockies, and includes the western portion of Saskatchewan and Alberta south of the North Saskatchewan. Between the 49th and 54th parallels it has an area of about 134,000 square miles, open prairie land for the most part in its southern portion, but wooded towards its northern and north-western confines. Its topography is still more diversified than that of the second plain, due, according to Dr Dawson, to longer and more energetic action of the

[1] *Handbook of Canada*, 1897.

denuding forces of rain and rivers, both before and after the glacial period. The character of the soil is still more varied than that of the second steppe; while there is much that is fertile and good, areas of some magnitude exist which without special methods cannot be profitably farmed.

Climatic conditions.

Considered broadly, the summers are characterized by high day temperatures and an abundance of sunshine, the winters by clear, very cold weather. Usually spring advances very rapidly, for though the mean temperature in Manitoba during April and May may be in the neighbourhood of 35°, the daily maximum would be at least 10° to 12° higher. The annual precipitation over the whole area is comparatively light, but is somewhat greater for the first than for the second and third prairie levels. In a general way we might say that the rainfall becomes somewhat lighter as we proceed westward. The greater part of the rain over the district, however, falls during the growing season, and hence is particularly effective agriculturally. The distribution is, for the most part, well adapted to the production of the finest quality of wheat. Mr Stupart[1] remarks that the fact should not be lost sight of that although the total annual precipitation only averages 13·35 inches for the territories (now the provinces of Saskatchewan and Alberta) and 17·34 inches for Manitoba, the amounts falling between April 1st and October 1st are respectively 9·39 inches and 12·87 inches, or 70·3 and 74·2 per cent. of the whole. The average 12·87 inches in Manitoba is not far short of the average for Ontario during the same six months[2].

Area suitable for farming.

It is estimated that there are in the three western provinces about 180,000,000 acres suitable for cultivation, the greater part of which is adapted to wheat growing. Of this area probably not more than 6 per cent. is at present under cultivation. A further territory to the north of Alberta and Saskatchewan, within the boundaries of Mackenzie, Keewatin, Ungava, and Yukon, contains more than 900,000,000 acres, and it has already been shown that wheat can be successfully grown at several points within this immense area.

Western agricultural problems demanding Soil Analysis.

The types of soils in the prairie region, as might be supposed, are not so numerous as in Eastern Canada or British Columbia, but no

[1] Director of the Canadian Meteorological Service. [2] *Handbook of Canada*, 1897.

systematic, comprehensive laboratory examination has been made. The general high fertility of these soils has rendered unnecessary for the most part assistance from the chemist in their management. Only in certain specific cases have we made analysis of these soils, to ascertain if a district were affected with alkali, to learn if the failure reported were due to an insufficient rainfall or to poverty of the soil, and occasionally to furnish information regarding the character of the soil in some new district about to be opened up for settlement. From the soils so examined, probably some 200 samples, I have selected a few as representative of large, uniform areas of virgin, *i.e.* uncropped, unmanured land, together with one or two samples of cultivated soils.

Supplementary to the analytical work done with the view of ascertaining the amounts of total and available plant food present, we have studied to some extent the following questions relating to north-western agriculture: methods of culture as affecting conservation of soil moisture; nitrification and exhaustion of fertility through continuous grain growing; the effect of irrigation on the plant food content of the soil.

Characteristics of Prairie Soils.

Humus and nitrogen content.

The distinguishing characteristic of the western prairie soils is the large proportion of vegetable matter intimately incorporated with the sand and clay. To this they primarily owe their remarkable fertility and lasting quality. For the most part, they contain abundant stores of the mineral elements of plant food, but no more than many Canadian soils of less productiveness in other parts of the Dominion. But we have invariably found that soils of great productiveness are characterised by large percentages of organic matter and nitrogen, and, on the other hand, that worn, or partially exhausted soils, resulting from continuous grain growing or other irrational treatment, and soils from naturally poor areas, show meagre amounts of these constituents.

We have, further, noticed, as far as soils in humid and semi-humid districts are concerned, that there exists a relationship between the organic matter and the nitrogen—that methods of culture which increase the amount of the former raise the percentage of the latter, and on the other hand when the organic matter is destroyed, nitrogen is dissipated.

Functions of Humus. Humus is not only a storehouse for nitrogen that may be readily nitrified and made available for crop use; it also

liberates during its decay goodly proportions of potash, phosphoric acid and lime. In all probability it furnishes a large part of the soil food supply of the growing crop.

Its influence on the physical condition of the soil is most markedly shown in increasing the capacity of the soil for holding moisture. Our investigations have shown that soils of the same type from adjoining areas, apparently under the same climatic conditions and with equal drainage, will retain moisture in proportion to the organic matter content. During the growing season prairie soils may retain amounts of water far in excess of those present in soils less rich in organic matter though favoured with a heavier precipitation—as in Eastern Canada. Further, the high absorptive capacity of these soils under suitable cultural methods allows moisture to be held over from one season to another, and thus it is possible in districts of scanty precipitation, by means of a fallow, to secure two good crops in three years when only very meagre yields would be obtained if the land were seeded every year. Humus also favourably modifies the tilth and temperature of both clays and sands.

Biologically, we have unfortunately no data to offer respecting these prairie soils except as to nitrification, but there can be but little doubt that a distinct relationship exists between the organic matter content and the bacterial life of the soil.

Nitrogen as an index of fertility. Our experimental work with soils *in situ* has assured us that of all the elements of plant food, nitrogen is the most potent in its influence on crop production. A high nitrogen content in soils of humid and semi-humid districts is invariably associated with a goodly proportion of humus-forming material and it is difficult to ascribe to each its own proper share in affecting the yield. But for prairie soils, whether clays or sandy loams, nitrogen may be regarded as the most reliable measure of their crop producing power. The extraordinary growth that characterises vegetation on the prairie as soon as the season opens is unquestionably due, for the most part, to the very rapid nitrification in the spring and early summer months consequent upon the large water content of the soil and the high temperatures which then prevail.

Causes of fertility.

The richness of these prairie soils lies in the tremendous accumulation of nitrogenous organic matter with its associated mineral constituents—the remains of countless generations of plant life and bacterial

activity. Since the glacial period these prairies have been continuously clothed with grasses and leguminous herbage. The generally level character of the region has precluded those losses of soil by erosion which naturally occur in more or less mountainous districts.

Peculiarly favourable climatic conditions for soil enrichment have existed and still exist in the north-western provinces. High diurnal temperatures, long days, and a sufficient rainfall during the growing season are conducive to a most luxuriant growth. Rapid nitrification and conversion of inert mineral matter into available plant food take place practically throughout the summer—and, withal, there is no excess of rain to leach out and carry off the soluble constituents[1]. These conditions, further, tend to the production of more or less soluble mineral matter, alkaline in character, largely carbonate of lime, which renders the soil favourable for bacterial activity and vegetable life in general, and probably is of assistance in the formation and conservation of humus[2]. And lastly, we have the winter season with its intense cold practically locking up the stores of plant food from the autumn until the season again opens. Waste from leaching, such as occurs in countries in which the winter is mild and open, is thus prevented. This important fact has been, for the most part, overlooked by those who have written upon the various problems of western agriculture.

Manitoban Soils.

As illustrative of the soils of the first steppe—the prairie of the Red River Valley—we have tabulated the results from a few typical examples, restricting the data to the more important constituents. As already stated, the plateau south of the Winnipeg group of lakes is of remarkable uniformity and the data of soil No. 1 are representative of a very large area of the immediate valley of the Red River, though perhaps not typical in all details of the whole plateau. It is a deep, black clay

[1] And here, perhaps, the opportunity best presents itself to say a word in reply to the question frequently asked as to the probable necessity of using superphosphate or other mineral fertilizers in the north-west. At present, at all events, there is no such necessity; over the larger portion of the prairie country, seasonal conditions undoubtedly to-day control the yields. As for the future, our work and observations lead us to believe that if the humus content of the soil is well maintained the day is far distant when there will be any need for phosphatic and potassic fertilizers.

[2] In speaking of the invariably alkaline reaction of prairie soils, it may be remarked that their black colour—which undoubtedly is an important factor in their absorption of heat as soon as the season opens—results in all probability from the action of the alkaline compounds referred to on the organic matter and is not due to the presence of finely divided carbon from prairie fires as advanced by the late Dr Geo. M. Dawson.

loam, of a fine and peculiarly characteristic granular structure. In the air-dried condition, it reduces easily to a greyish brown or greyish black powder. Though there is present a considerable amount of undecomposed root fibre, the soil proper presents a remarkable homogeneity in appearance, indicating a process of physical refining in its formation and a uniformity in chemical composition. The very large amount of organic matter present is undoubtedly intimately incorporated with the clay and sand which constitute the basis of the soil.

Though containing a large amount of clay, laboratory experiments show that this soil does not readily "puddle" on moistening, nor on subsequent drying does it form into a hard mass, but granulates on moderate pressure. The large amount of organic matter present has already been remarked; it exceeds 25 per cent. of the water-free soil. The nitrogen, calculated on the same basis, is found to be practically 1 per cent., from which it may be estimated that there is contained in an acre of soil to the depth of one foot from 20,000 to 25,000 pounds at least of this element. Since ordinary fertile soils to a like depth contain from 3500 to 10,000 pounds, the vast reserve of this valuable constituent in this prairie soil is apparent.

The soil is also very rich in potash, containing an amount (1·033%) far in excess of that ordinarily met with in the fertile soils of Eastern Canada. Our data have indicated that good agricultural soils possess usually between 0·25 and 0·5 per cent. of potash.

Of phosphoric acid, it contains 0·29 per cent. This is slightly above the average, most of our good soils showing between 0·15 and 0·25 per cent.

The fairly large percentage of lime is worthy of note, since it indicates not only a fair supply for crop use but also a condition of the soil that should be particularly favourable to nitrification.

This prairie land, as regards the elements of fertility, ranks with the richest of known soils.

The late Dr Geo. M. Dawson wrote some years ago: "Of the alluvial prairie of the Red River much has already been said, and the uniform fertility of its soil cannot be exaggerated. The surface, for a depth of two to four feet, is a dark mould, composed of the same material as the subsoil, but mingled with much vegetable matter. Its dark colour is no doubt due in part to the general accumulation of the charred grasses left by the prairie fires. The soil may be said to be ready for the plough, and in turning the tough thick prairie sod, the first year a crop of potatoes may be put in,

though it is not efficiently broken up till it has been subjected to a winter's frost. When the sod has rotted, the soil appears as a light, friable mould, easily worked and most favourable to agriculture. The marly alluvium underlying the vegetable mould, would in most countries be considered a soil of the best quality, and the fertility of the ground may, therefore, be considered as practically inexhaustible.

"The area of this lowest prairie has been approximately stated as 6900 square miles, but the whole is not at present suitable for agriculture. Small swamps are scattered pretty uniformly over its surface. The greater part of these swamps are, however, so situated as to be easily drained, either into the Red River or some of its tributaries, which are usually depressed 30 or 40 feet below the level of the surface."

Soils Nos. 2 and 3 are from Portage la Prairie, a district lying some 50 miles directly west of Winnipeg. It is one of the earliest settled localities in the north-west, and has long enjoyed a reputation for producing wheat of the very highest quality. In No. 2 we have an example of the virgin prairie—uncropped and unmanured; in No. 3, the same soil after 25 years of cultivation, in which grain growing was interspersed with fallowing to clean the land. The virgin soil shows more root fibre than the cropped soil and is somewhat darker in colour. Both might be described as black, friable loams, containing a considerable proportion of sand. The analytical data afford evidence of their richness in the elements of plant food, though they are not quite equal to the soil from the Red River Valley either in "total" or "available" constituents.

A comparison may be made of Nos. 2 and 3, since it is of much interest to learn what effect grain growing for a number of years may have had on the composition of the soil. A considerable reduction will be noticed in the percentages of organic matter and nitrogen in the cultivated soil due, as will be shown later, in a very large measure to fallowing—a system of immense value for the conservation of moisture and the destruction of weeds, but particularly wasteful of organic matter and nitrogen. In the mineral constituents no great differences are to be observed—the losses so far as they may be gauged by chemical analysis have not been at all excessive. This is not to be wondered at as the wheat crop does not remove large amounts of plant food in such a period as 25 years, representing say 16 crops.

Nos. 4 and 5 are composite samples from the Experimental Farm,

Brandon, about 130 miles west of Winnipeg. They resulted from monthly collections (May to November) from plots under different cultural treatments in connection with moisture conservation experiments. In so far as physical character is concerned these two samples are practically identical, the soil being a mellow, black loam of a somewhat sandy type.

The tabulated data bear out their similarity in composition and we may regard them as typical and illustrative of the true prairie soil. We have only to remark the abundance of vegetable matter, the high nitrogen-content and the liberal supply of the mineral elements, and more particularly of potash and lime.

No. 6 is a soil from the district immediately west of Lake Dauphin and north-west of Lake Manitoba. The area is one that in parts is covered with willow and other "scrub," necessitating clearance before cultivation. This soil is probably to be regarded as representative of those lands immediately surrounding the lakes and subject to more or less flooding during the early part of the season, for which drainage is of course necessary. It is a sandy loam, rich in organic matter, but with sufficient clay to render it somewhat refractory on drying. When drained it proves suitable for wheat growing, excellent returns having been obtained in favourable seasons.

Nos. 7 and 8, the remaining two samples, are black, sandy loams from the Valley River, Dauphin district, collected in 1906 in an investigation to learn the influence of environment on the composition of wheat—a matter still under study in the Farm Laboratories. The richness of these loams in organic matter and their high nitrogen-content is worthy of remark. In potash, they are decidedly poorer than the stronger or more clayey soils of the north-west—indeed, in this constituent they are somewhat below the average found for Canadian soils of medium fertility. The percentages of "available" potash are similarly low, though not reaching the limit set by Dyer as indicating the need of a potassic fertilizer.

The amounts of phosphoric acid are considerably lower than in the prairie soil of the Red River Valley, but are about equal to those present in soils of average fertility. The large proportion of lime in these soils would undoubtedly favour rapid nitrification and also serve to render effective the somewhat sparse supply of phosphoric acid.

In the examples discussed two distinct types of Manitoban soils are represented, the heavy clay loam covering the true prairie region

Soils from Manitoba.

Results of Analysis (calculated to the water-free basis).

No.	Locality	Character of Soil	Organic and volatile matter (loss on ignition) %	Nitrogen %	Phosphoric acid[1] (P_2O_5) %	Potash[1] (K_2O) %	Lime[1] (CaO) %	Available constituents[2]		
								Phosphoric acid (P_2O_5) %	Potash (K_2O) %	Lime (CaO) %
1	Red River Valley, near Morris	Virgin prairie soil—black heavy clay loam	26·29	1·005	·288	1·033	1·89	·054	·076	·581
2	Portage la Prairie	,, ,, ,, black sandy loam ...	19·43	·651	·178	·658	1·05	·038	·056	·529
3	,, ,, ,, ...	Prairie soil, cropped for 25 years.....	14·79	·506	·170	·588	1·61	·033	·048	·776
4	Brandon	,, ,, black loam, rather sandy ...	11·27	·346	·123	·819	1·14	·029	·057	·572
5	,, ...	,, ,, ,, ,, ,, ,, ...	12·05	·431	·136	·841	1·02	·027	·076	·462
6	Dauphin, Dauphin District ...	Black sandy loam	11·44	·363	·215	·687	1·89	·023	·018	1·121
7	Valley River, Dauphin District	,, ,, ,, ...	21·54	·662	·155	·144	10·57	·007	·017	1·346
8	,, ,, ,, ,, ,,	,, ,, ,, ...	13·11	·379	·133	·194	3·54	·007	·007	·949

[1] The solvent used in the determination of the "total" percentages of phosphoric acid, potash and lime was hydrochloric acid, sp. gr. 1·115, 10 grams of the air-dried soil being digested with 100 c.c. of the acid at the temperature of the water bath for 10 hours.

[2] In the estimation of the "available" constituents, 1 per cent. citric acid solution was employed, digesting 100 grams of air-dried soil with 1000 c.c. of the solvent for seven days at room temperatures.

to the south of the province, and undoubtedly one of the finest wheat soils in the world, and the other representative of the sandy loams of the north-western and more humid area, more or less covered with small trees and shrubs—a district regarding which we know less as to suitability for wheat growing, but nevertheless one which has produced profitable crops. Considered as a whole the quality of the wheat of this north-western section has not been equal to that of the southern and more distinctly prairie portion of the province, but there is evidence to support the view that the grain will improve in character with drainage and further cultivation of the soil.

Dr Russell has made mechanical analyses of the soils and reports on them as follows :—

Manitoban Soils.

Locality	Red River Valley	Brandon	Portage la Prairie	Portage la Prairie 25 years cropped	Dauphin District	
Number of Sample	1	4	2	3	7	8
Soil :						
Fine gravel[1], above 1 mm.	—	—	—	—	—	—
Coarse sand, 1 to 0·2 mm.	1·6	2·5	4·3	11·4	21·1	19·5
Fine sand, 0·2 to 0·04 mm.	3·8	15·4	8·6	8·4	22·6	33·3
Silt, 0·04 to 0·01 mm.	17·1	17·7	30·0	29·6	10·7	11·0
Fine silt, 0·01 to 0·002 mm.	28·2	16·1	15·6	14·5	6·4	6·2
Clay, below 0·002 mm.	23·3	29·2	16·5	14·2	3·4	4·1
Loss on ignition	26·3	11·3	19·4	14·8	21·5	13·1

"The Red River Valley soil shows the rather unusual feature of possessing more fine silt than clay; in mechanical composition it resembles the weald clay soils of Kent. But whilst the weald soils are difficult to work and are indeed commonly in pasture the Red River soil works very easily. The difference is due to the large amount of organic matter uniformly distributed throughout the soil and to the presence of a sufficient amount of lime. We have here an interesting case of a soil made workable by organic matter; so long as this does not fall too low the soil may be expected to maintain its fertility, but if continuous cereal cropping were persisted in too long without

[1] As the soil had already gone through a 1 mm. sieve the fine gravel could not be determined.

replacing any organic matter the soil would become difficult to cultivate. The Brandon soil is somewhat similar.

"The Portage la Prairie soil closely resembles the good brick earths of the south-east of England. It is not dependent for its tilth on the organic matter; nevertheless the supply of organic matter should be kept up because it constitutes a rich source of nitrogenous plant food.

"The Dauphin District soils are very light and deficient in clay. Without their organic matter they would probably become like soils of similar mechanical composition elsewhere—lean, hungry sands, excellent for market gardening if well manured, but poor farming soils. Here, too, then we have a case where the mechanical structure of the soil is profoundly modified by the organic matter present."

Saskatchewan Soils.

In reviewing for the purposes of this paper the Saskatchewan soils examined by us during the past twenty years, a difficulty has been encountered in selecting only those truly representative of fairly large areas, since the second prairie steppe, comprising the larger part of this province, is not characterized by the uniformity noticed in the Red River Valley. This fact precludes the possibility of presenting here examples of all the types to be found, but it is worthy of remark, that the larger number of the soils examined—and more particularly those in the noted wheat-growing districts—have been found to be abundantly supplied with humus-forming material and nitrogen.

No. 1. A rich, black loam from Moosomin, a point on the main line of the C.P.R., 220 miles west of Winnipeg. The elevation of this locality is about 1800 feet, and this soil may be regarded as fairly representative of the south-eastern part of the second prairie level. As in the types we have considered from the first steppe, this true prairie soil possesses abundant stores of plant food and is, judged by accepted standards, one of high fertility. It has not, however, looked at simply from the chemical point of view, a rank equal to that from the valley of the Red River.

No. 2. From the district of Tisdale, on the Canadian Northern Railway, about 160 miles due north of Indian Head. The district is in a large measure comparable with the Dauphin country already described, being partly wooded with scrub, poplar, &c., and therefore, unlike the true prairie, requiring clearance. The soil is a greyish black

THE PROVINCES OF MANITOBA, SASKATCHEWAN AND ALBERTA, CANADA.

Showing prairie and wooded areas and the lines of the first and second steppes.

loam of a decidedly clayey nature, containing almost 0·5 per cent. of nitrogen, with notable amounts of potash and lime.

Nos. 3 and 4 are from Saltcoats and Yorkton, points on the north-western branch of the C. P. R., 250 and 270 miles, respectively, west of Winnipeg, and approximately 75 miles north-east of Indian Head. Both are black, sandy loams of the true prairie type, rich in vegetable matter and nitrogen with excellent percentages of phosphoric acid and potash.

Nos. 5 and 6 are black loams of a markedly sandy character, taken from areas that had been under grain (without manure) for a period of about 15 years. Wolseley, the place of the collection, is about 20 miles east of Indian Head on the C. P. R. and has produced large crops of very fine wheat. These soils have borne probably ten crops of grain, with a bare fallow every third summer, but they are still of an exceedingly rich character, plentifully supplied with semi-decomposed vegetable matter and high in nitrogen—indeed as regards these constituents the data do not differentiate them from virgin prairie soils. They are decidedly above the average in "total," but not in "available" phosphoric acid.

Nos. 7 to 10 inclusive are from the Dominion Experimental Farm, Indian Head, and constitute a very instructive series, since they allow a comparison between the virgin pariric with the same soil after 22 years of cultivation, without manure. The soil would be designated as a heavy clay loam. A complete record of the cropping and fallowing since the prairie was broken in 1882, shows that the "cultivated" soil had borne six crops of wheat, four of barley and three of oats, with a fallow between each crop since 1887, nine fallows in all. The virgin soil was taken from an adjacent area about 150 feet distant from where the cultivated soil had been taken. The samples were of a composite character and every precaution was taken to have them thoroughly representative. There is every reason to suppose that the soil, over the whole area examined, was originally of an extremely uniform nature; in other words, that at the outset the nitrogen-content was practically the same for the soils now designated as virgin and cultivated, respectively. The tabulated data show the percentage of organic matter and plant food in the first four and the first eight inches of these soils, and make very clear that enormous losses of organic matter and nitrogen have followed upon the present method of continuously cropping with grain. The particulars respecting the nitrogen are given in the following arrangement, which allows a ready comparison of the two soils in this important matter.

Depletion of Soil Nitrogen.

Nitrogen-content of virgin and cultivated soils, Indian Head, Sask.

	To a depth of 4 inches		To a depth of 8 inches	
	Per cent.	Lbs. per acre	Per cent.	Lbs. per acre
Virgin soil	·409	3824	·371	6936
Cultivated soil	·259	2421	·254	4750
Difference or loss due to removal in crops and to cultural methods	·150	1403	·117	2186

Though the cultivated soil to-day, after nearly a quarter of a century's working, is still very rich and possibly might yield as fine a crop as it did at the outset, yet, compared with the untouched prairie, it is seen to have lost practically one-third of its nitrogen.

An enquiry as to what proportion of this loss is due to removal by crops and what to cultural operations shows that the nitrogen contained in the various grain crops grown in the 22 years amounted to approximately 700 lbs. per acre. If we subtract this amount from the total loss, calculated to a depth of 8 inches of soil, we shall see that the amount of nitrogen dissipated by methods of cultivation is more than twice as great as that removed in the crops. The loss ordinarily in the grain growing districts of the north-west would not be in all probability as great as that here recorded, because as a rule the land is fallowed every third year only. Nevertheless the deterioration must be marked, and, unless checked by the adoption of a system of rotation involving the formation of a sod, and the keeping of stock, will inevitably lead to that low productiveness which now characterizes large areas in eastern North America. A study of these partially exhausted areas both in Canada and in the north-eastern States makes it clear that the deterioration has been due, in a very large measure, to the loss of humus and the dissipation of nitrogen consequent upon growing grain and potatoes without any due return of organic matter.

A marked falling off in phosphoric acid is also to be noted, though what is perhaps of more significance is the reduction in the proportion of this element in the available condition. Since loss of phosphoric acid cannot be accounted for save in removal by crops, it

Soils from Saskatchewan.

Results of Analysis (calculated to the water-free basis).

No.	Locality	Character of Soil	Organic and volatile matter (loss on ignition) %	Nitrogen %	Phosphoric acid (P_2O_5) %	Potash (K_2O) %	Lime (CaO) %	Available constituents		
								Phosphoric acid (P_2O_5) %	Potash (K_2O) %	Lime (CaO) %
1	Moosomin	Black loam	11·79	·479	·116	·306	·95	—	—	—
2	Tisdale	Greyish-black loam	14·23	·480	·202	·622	1·11	·024	·041	·568
3	Saltcoats	Black, sandy loam	13·54	·572	·213	·340	2·89	·018	·033	1·110
4	Yorkton	,, ,, ,,	14·01	·504	·211	·496	1·17	·025	·048	·531
5	Wolseley, N.E. ¼, Sec. 27	Black loam (cultivated)	13·93	·514	·391	·555	·87	·005	·011	·306
6	,, S.W. ¼, Sec. 27	,, ,, (,,)	10·98	·389	·369	·512	·76	·005	·018	·264
7	Indian Head	Black clay loam. Taken to a depth of 4 inches	13·31	·409	·212	·863	1·26	·036	·070	1·187
8	,, ,,	,, ,, ,, Taken to a depth of 8 inches	12·83	·371	·234	·868	1·41	·032	·059	1·261
9	,, ,,	,, ,, ,, Taken to a depth of 4 inches (cultivated)	10·20	·259	·159	·839	3·44	·016	·039	1·384
10	,, ,,	,, ,, ,, Taken to a depth of 8 inches (cultivated)	10·70	·254	·163	·898	3·51	·013	·038	1·336
11	Vermilion Hills, Tp. 21, R. 5, W. 3rd.	Dark brown sandy loam	10·43	·354	·164	·164	·50	·044	·050	·383
12	Maple Creek, Sec. 16, Tp. 11, R. 26, W. 3rd.	Heavy clay loam	5·54	·134	·064	·300	1·06	—	—	—

would seem that in continuous grain growing the rate of abstraction exceeds that of conversion.

In the "total" potash the differences throughout the series are not large, but, as in the case of phosphoric acid we find that the percentage "available" in the cultivated soil is considerably less than in that of the prairie[1].

No. 11 from the prairie in the neighbourhood of Vermilion Hills, 130 miles west of Indian Head and some 20 miles north of Lake Chaplin. It is a dark-brown, sandy loam. In organic matter and nitrogen it is fully the equal of the heavier (clay) loams of the prairie, but as regards phosphoric acid, potash and lime, it is, as might be expected, somewhat inferior. Although the "total" stores of this mineral plant food may not be very large it is significant that the "assimilable" proportions are not less than in those heavier loams which are considered wheat soils par excellence.

Dr Russell has made mechanical analyses of the soils and reports on them as follows:—

Saskatchewan Soils.

Locality	Wolseley	Tisdale	Indian Head	
Number of Sample	5	2	8	a[2]
Soil:				
Fine gravel[3], above 1 mm.	—	—	—	—
Coarse sand, 1 to 0·2 mm.	16·7	0·9	10·4	10·2
Fine sand, 0·2 to 0·04 mm.	14·8	24·1	13·7	9·9
Silt, 0·04 to 0·01 mm.	27·7	20·5	15·3	15·3
Fine silt, 0·01 to 0·002 mm.	8·1	13·7	11·9	11·1
Clay, below 0·002 mm.	15·1	21·3	27·2	33·9
Loss on ignition	13·9	14·2	12·8	16·8

"The Wolseley soil shows a remarkably uniform distribution of the various grades of particles. In its general structure it resembles the Portage la Prairie soil, and the remarks made on p. 346 apply here also.

[1] There is at times a certain loss of surface loam in the older cultivated areas by drifting, and this in some cases would affect the phosphoric acid and potash content, and more especially that portion which is available.

[2] A composite sample not dealt with in the table of chemical analysis.

[3] As the soil had already gone through a 1 mm. sieve the fine gravel could not be determined.

"The Tisdale soil owes its clay-like nature partly to the absence of coarse sand and partly to the rather large quantity of clay present. It contains no mineral material capable of keeping it open and friable, but its abundance of organic matter serves this purpose instead.

"The Indian Head soil contains a large amount of clay which, however, is tempered by the presence of 10 per cent. of coarse sand. It would still be rather intractable were it not so well supplied with organic matter and lime."

No. 12 is from an area in the eastern part of the third steppe, 281 miles west of Indian Head along the main line of the C.P.R. and not far from the boundary between Saskatchewan and Alberta.

The district from which this soil was taken enjoys as a rule but a very limited rainfall and, previous to the adoption of special methods for the conservation of moisture, gave but scanty yields. It was thought by some that the poor crops were due to a deficiency in some important fertilizing constituent, or to the presence of "alkali" or other matter deleterious to plant growth. Analysis shows that there is no lack of plant food, though the percentages of organic matter and nitrogen are only about one half of those found in the richer prairie soils. Absence of "alkali" was established, and the conclusion reached that the meagre yields were due to insufficient moisture rather than to any inherent fault in the soils.

Albertan Soils.

No. 1. This soil, a black, sandy loam, was collected in the neighbourhood of Tilley, a point on the main line of the C.P.R. about 50 miles west of Medicine Hat, a district that owing to sparse rainfall has hitherto been considered better adapted to ranching than to grain growing. As in the case of the two immediately preceding examples this soil was supposed to be deficient in some particular or to contain alkali. The data, however, show that there is an abundance of plant food present and an entire absence of alkali. Improved methods of culture, resulting in the better conservation of soil moisture, have shown that the poor yields were not due to the poverty of the soil, but to insufficient water supply.

No. 2, from the Dominion Experimental Farm at Lethbridge, an important centre in Southern Alberta, a true prairie region, where until recently ranching has been the chief branch of agriculture. Irrigation is desirable, if not indeed necessary, but in many seasons fairly good yields can be obtained by the adoption of proper cultural methods for

the conservation of soil moisture. The soil appears to be extremely uniform in character and very productive provided there is a sufficiency of moisture.

The sample, taken in this case to a depth of 12 inches, is a dark grey, inclining to black, sandy loam, light and friable, free from stones and containing an abundance of root fibres. Though not as rich in organic matter and nitrogen as the majority of the prairie soils hitherto considered, the results are quite satisfactory, especially when the greater depth to which this sample was taken is considered[1]. In mineral constituents it seems to be fairly well supplied, the amounts being such as are possessed by many soils of high productiveness.

No. 3 was collected from an uncultivated area on a bench in the valley of the Elbow River, some few miles from Calgary. The soil of the district is stated to be "well fitted for either cultivation or grazing." It might be classed as a light to medium, black, prairie loam, especially rich in organic matter. It is practically neutral and is well supplied with plant food.

Soils 4 and 5 were taken at no very great distance from No. 3 and are in appearance very similar to it. They were examined to learn what effect irrigation might have on the stores of fertility. No. 4 is from a non-irrigated area while No. 5 is from irrigated land, collected 50 feet from the lower side of an irrigation ditch and 100 feet from No. 4.

Undoubtedly the feature of greatest interest in the comparison of the data is the decidedly higher percentages of soluble (available) mineral constituents in the irrigated soil, and it is important to note that, while the non-irrigated land is neutral, the irrigated soil is slightly alkaline. These features are not uncommon and two possible causes for them may be advanced. The first is the deposition of mineral salts from the irrigation water and the second—probably the chief cause—is the bringing up of these compounds from the lower strata by increased capillarity induced by greater surface evaporation consequent upon irrigation.

It may be said that as Southern Alberta is of the true prairie character, so Northern Alberta is largely wooded, enjoying a more liberal rainfall and is naturally a country better adapted to mixed farming. The soils of Northern Alberta are for the most part characterized by high percentages of organic matter and nitrogen and in this

[1] In all instances, unless otherwise specified, the soil collections were made to a depth of 9 inches.

Soils from Alberta.

Results of Analysis (calculated to the water-free basis).

No.	Locality	Character of Soil	Organic and volatile matter (loss on ignition) %	Nitrogen %	Phosphoric acid (P_2O_5) %	Potash (K_2O) %	Lime (CaO) %	Available constituents		
								Phosphoric acid (P_2O_5) %	Potash (K_2O) %	Lime (CaO) %
1	Tilly, Tp. 16, R. 13, W. 4th...	Sandy loam	11·12	·398	·174	·266	·37	——	——	——
2	Lethbridge (1st foot)	Dark grey, or black sandy loam	5·89	·215	·123	·462	1·04	·008	·029	·959
3	Calgary, N.W. ¼, Sec. 21, Tp. 23, R. 1, W. 5th.	Black granular sandy loam	13·69	·530	·210	·520	·71	·009	·035	·498
4	,, S.W. ¼, Sec. 15, Tp. 23, R. 1, W. 5th.	,, ,, ,, ,, (non-irrigated)	16·12	·549	·240	·380	·90	·004	·028	·440
5	,, ,, ,, ,,	,, ,, ,, ,, (irrigated).....	15·30	·574	·180	·380	1·28	·012	·035	·568
6	Innisfail (1st foot)	Black sandy loam	12·09	·403	·155	·384	·68	·016	·015	·392
7	Lacombe, 1 inch to 8 inches...	,, ,, ,, ...	8·78	·326	·136	·250	·63	·023	·024	·385
8	Lac la Nonne	,, ,, ,, ...	17·63	·673	·190	·611	1·00	·037	·022	·584
9	,, ,, ...	,, ,, ,, ...	14·34	·514	·197	·673	1·24	·050	·035	·799

respect are somewhat superior to those in the southern part of the province. We have in this a certain confirmation of the view that a relationship exists between rainfall and the organic content of the soil.

The samples so far considered from this province have been representative of areas in Southern Alberta, the remaining examples are from points north of Calgary.

No. 6 is from Innisfail, an excellent district for dairying and mixed farming, some 80 miles north of Calgary on the Edmonton branch of the C.P.R. This sample had been collected to a depth of 12 inches. As received, in the air-dried condition, it was a loose, friable, greyish black, sandy loam, full of fibre and evidently rich in organic matter.

No. 7 is fairly representative of the soil on the recently acquired Dominion Experimental Farm at Lacombe, a point some 40 miles north of Innisfail. The country and soil in this neighbourhood are similar in character to those of the Innisfail district, just described, and indeed may be considered typical of a very large part of this northern portion of the province.

Nos. 8 and 9 are clay loams from Lac la Nonne, a district lying some 40 miles north-west of Edmonton. These loams are very similar, containing a large proportion of clay and organic matter; they are greyish black in colour when air-dried. They are rich in nitrogen, above the average in potash and lime, and are fairly well supplied with phosphoric acid. Under proper cultural operations and favourable climatic conditions, they should prove to be highly productive soils.

Dr Russell has made mechanical analyses of the soils and reports on them as follows:—

Alberta Soils.

Locality	Lethbridge (1st foot)	Calgary	Innisfail	Lac la Nonne
Number of Sample	2	3	6	9
Soil:				
Fine gravel[1], above 1 mm.	—	—	—	—
Coarse sand, 1 to 0·2 mm.	22·4	8·5	6·5	0·1
Fine sand, 0·2 to 0·04 mm.	25·0	26·7	16·4	10·6
Silt, 0·04 to 0·01 mm.	11·3	17·1	32·0	20·5
Fine silt, 0·01 to 0·002 mm.	11·0	11·4	10·0	21·8
Clay, below 0·002 mm.	17·0	17·0	15·3	24·6
Loss on ignition	5·9	13·7	12·1	14·3

[1] As the soil had already gone through a 1 mm. sieve the fine gravel could not be determined.

"The soils are all well supplied with the fine particles that hold water near the surface for the crop, and are therefore adapted to the dry conditions in which they occur. The Lethbridge soil contains sufficient coarse sand to render cultivation easy. On the other hand, the Lac la Nonne soil is almost devoid of this constituent and depends for its workability on the organic matter present. The Calgary and Innisfail soils have the structure of good loams, and, like the Portage la Prairie soil on p. 346, are not dependent for their tilth on the organic matter, although they derive much plant food from this source."

Conservation of Soil Moisture.

It will be evident from the facts brought forward in this paper that while it is advisable to adopt such a system of farming as will lead to the maintenance of fertility, the necessity of returning plant food in manures and fertilizers will not be generally felt for some time to come, so rich is the soil of the prairies over very large areas. But while, as yet, nitrogen (or any other element of fertility) cannot be regarded as the limiting factor, the amount of soil moisture available during the growing season does most markedly affect the yield. We consequently find that the important question of prairie farming, and more particularly in districts of sparse rainfall, is the conservation of moisture for the crop's needs.

Fallowing is the general means adopted to this end. This comprises the preparation by deep ploughing of a reservoir, so to speak, for the storage of the rainfall in the soil, and the formation by frequent cultivation of a dry earth mulch to check evaporation. To ascertain the extent to which water may be carried over by fallowing from one season to another, a series of experiments was conducted some years ago on the Experimental Farms at Brandon and Indian Head, in which the amounts of moisture were determined to depths of 8 and 16 inches, respectively, on soils fallowed and cropped the previous season[1]. It was shown that at Brandon the soil which had been fallowed contained during May, June and July—the months of growth—amounts varying from 330 to 65 tons per acre, to a depth of 16 inches, over and above those in the soil that had borne a crop. Similarly at Indian Head the excess of moisture in the land that had been fallowed varied from 175 to 160 tons. While the amounts of moisture so conserved must depend upon the character of the season and the thoroughness with which the fallowing is carried out, the evidence furnished by this investigation is sufficient

[1] The data of this investigation will be found in the Report of the Chemist, Dominion Experimental Farms, 1900.

to show the great value of this practice as a means of storing up moisture for the crop of the succeeding year[1].

NOTES ON THE AGRICULTURE OF THE PRAIRIES.

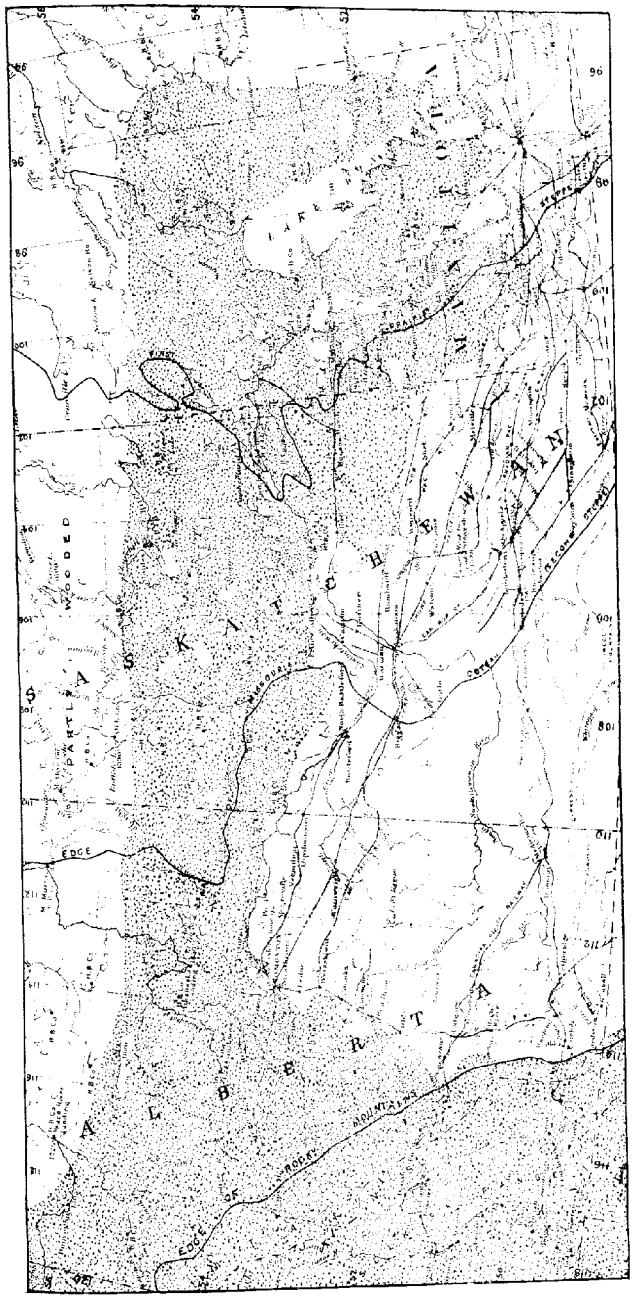
Manitoba. Grain growing has been and will probably remain the most important feature in this province and more especially in the Red River Valley proper. However, recent years have witnessed a change. More and more stock is being kept and the tendency of the future will be largely towards smaller holdings and mixed, *i.e.* diversified farming. Dairying and the production of beef, mutton and pork are already extensively prosecuted with profit in many sections. Grass, roots and all classes of forage crops can be grown successfully. Of the cereals, wheat is the staple, but oats, barley and flax are also largely sown.

The soils as we have seen are varied in character, from heavy clays in the Red River Valley to sandy loams in the northern parts, but all are richly endowed with vegetable matter and plant food, more particularly nitrogen. Many of these soils have now been cropped for 25 and 30 years and as yet without any apparent falling off in productiveness. In this province the rainfall is usually sufficient for the needs of the crop, more particularly if special methods for its conservation are followed.

Saskatchewan. Considering the province as a whole, the characterizing feature, as in Manitoba, is grain growing, chiefly wheat. There are however certain fairly well defined areas, each with its own more or less special adaptation to some particular branch of agriculture. Thus, for instance, in the western part cattle ranching on the large scale has been for many years the principal industry. A considerable proportion of the northern half of the province is as yet unsurveyed, but from such facts as have been gained, it will prove most suited to mixed farming.

As the province becomes more thickly settled the one-crop system (grain and fallow) is giving place to more rational methods and diversified farming is becoming more and more popular.

[1] Unfortunately, fallowing is not without its concomitant evils. We have already pointed out that dissipation of humus and nitrogen results from continuous grain growing and that the greater part of this loss is more particularly consequent upon the stirring and opening up of the soil by repeated cultivation during the fallow season. It must now be stated that a further loss may result from fallowing; viz. the removal of more or less of the rich surface soil by erosion and drifting. The constant cultivation of the land breaks up the fibre—the binding element which gives the toughness to the prairie sod. As the fibre becomes shorter the surface loam more readily dries and pulverizes; it is then easily carried away by the strong winds which prevail at certain seasons in prairie regions. Very serious losses have occurred from this cause in some of the older cultivated districts of the north-west. The adoption of a cropping system in which the soil is occasionally put in sod suggests itself as the natural and best corrective.



SKETCH MAP PREPARED BY GEOLOGICAL SURVEY OF CANADA.

Prairie, unshaded; Wooded, stippled; Area north of stippled, partly prairie and partly wooded.

Soils of several types are to be found, and wheat of excellent quality is apparently produced, alike on clay and sandy loams, provided climatic conditions are favourable.

It is as yet too early to notice any effect on the soil in the grain growing districts from the one-crop system, but as pointed out there is a marked destruction of the organic matter and dissipation of the nitrogen where such a plan is followed, and this eventually will injuriously affect the soil both chemically and physically.

Alberta. The world-wide reputation of Alberta as a typical ranching country has been well earned, though it is more particularly in the southern part of the province that this branch of agriculture has been followed. In more recent years the growing of winter wheat has in certain districts of the south largely displaced the raising and grazing of stock. Northern Alberta is more particularly adapted to mixed farming and for some years past, on the lines of railway, co-operative dairying has been profitably prosecuted.

Southern Alberta may be considered a semi-arid country and one therefore in which provision for irrigation is desirable. Mention may therefore be made of the extensive irrigation scheme of the Canadian Pacific Railway, by which about eleven hundred thousand acres immediately east of Calgary will eventually be watered. A survey of this territory by the writer, in 1906, showed some variation in the character of its soils, though like all true prairie areas, uniformity was the prevailing feature, the characteristic soil being a moderately heavy black loam from four to eight inches in depth, with a subsoil of chocolate coloured clay. The whole area appears to be one well adapted to diversified farming.

In concluding this review, we may be allowed again to emphasize the general uniformity of the prairie soils, their richness in plant food, more especially in nitrogen, and their favourable physical condition due, chiefly, to the large proportion of semi-decomposed vegetable matter they contain. Further, though the rainfall over a large portion of the prairies is not a generous one, as judged by Eastern standards, good yields may be obtained by fallowing, even in very dry districts. And lastly, the climatic conditions usually prevailing in the prairie country are such as to bring about a rapid conversion of the stores of plant food into available forms without undue waste, and to favour a luxuriant growth and early ripening of the crop.

The writer desires to record his thanks to Dr E. J. Russell, Goldsmith Chemist, Rothamsted Experiment Station, who, in addition to the work involved in making the mechanical analysis, has very kindly expressed his appreciation of the soils from the physical data.

HYDROLYSIS OF THE PROTEIN OF LINSEED.

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Every farmer who feeds stock finds the necessity for supplementing his home-grown foods, which are usually of a bulky nature containing large proportions of carbohydrate and fibre, by purchasing concentrated foods rich in nitrogen and fat. Experience shows that it is by no means a matter of indifference which concentrated food is selected to mix with any particular bulky food, and the trend of modern physiological chemistry seems to point to a definite explanation of this fact. According to modern views, the protein eaten by an animal is split normally by the digestive ferments into amino-acids and other crystalline nitrogenous substances. It is in the form of such comparatively simple substances that the animal absorbs its nitrogen from the alimentary canal, and from them that it builds up its own characteristic proteins.

Since it has been shown possible to separate the amino-acids formed from proteins by hydrolysis, our knowledge of the composition of proteins has greatly advanced. We now know that every protein has its own special composition which can be determined with some kind of accuracy by a study of the products of its hydrolysis. Many proteins have already been hydrolysed, amongst them a certain number of those entering into the composition of animals, and it appears that for the synthesis by the animal of its characteristic proteins, the amino-acids and other crystalline nitrogenous substances prepared by digestion of ingested protein are required in definite proportions.

The proportions in which these substances are yielded by hydrolysis of the proteins of many common foods have been determined and found to be very variable. Thus glutaminic acid results from the splitting of such animal proteins as casein, egg-albumin, serum-albumin and globulin, and milk-albumin, to the amount of from 8 to 10 per cent.

The proteins of the cereals, on the other hand, give from 30 to 40 per cent. of this substance whilst the proteins of the leguminous seeds yield only about half that amount.

Again, animal proteins appear to yield tryptophane on hydrolysis, whilst this substance is absent from the splitting products of some vegetable proteins, notably the zein of maize. This latter is of especial interest, for Willcock and Hopkins[1] have shown that tryptophane introduced into the diet of mice receiving zein as their only protein food, markedly increased their survival period. It is also a well-known fact that gelatin cannot entirely replace protein in the diet of an animal, and this is due to the absence of certain necessary groups, e.g., tyrosine, in its composition.

It appears, therefore, reasonable to assume that a food whose protein constituents are deficient in one or more of the groups necessary for animal nutrition will give the most economical results when combined in a diet with foods whose protein constituents yield on digestion abundance of these splitting products. From this it follows that a knowledge of the products of hydrolysis of the proteins of the common foods used on the farm may be expected ultimately to provide data which will form a scientific basis for the compounding of food rations.

It is with this object in view that the writer undertook the examination of the proteins of linseed, a feeding stuff on which the farmers of Great Britain spend annually many thousands of pounds.

Preparation of the linseed for extraction.

The best seed obtainable was sifted and screened free from impurities and dust, and ground in a mill to as fine a meal as possible. The fat was removed by repeated leachings with the distillate obtained from ordinary petrol up to 85° C. The meal was freed from remaining petrol by exposure to air, and the husk removed by rubbing through a sieve. The fine powder thus obtained was used for extraction of the protein.

Osborne[2] has already studied the proteins of linseed. He finds that the greater part of its nitrogen exists as a globulin soluble in 10 per cent. sodium chloride solution, from which it can be separated by dialysing. In addition to this he has also shown that linseed contains a protein insoluble in neutral salt solutions, which can be extracted by 0·2 per cent. potassium hydrate solution. He also notes the presence of

[1] *Journal Physiol.* Vol. xxxv. Nos. 1 and 2, Dec. 29, 1906.

[2] *Amer. Chem. Journ.* Vol. xiv. 1892, p. 629.

several other protein substances, which he considers to be derived from the globulin. The results of his investigation also show "that the globulin and the body extracted by dilute potash are the only proteins found in the extract which are precipitated from an alkaline solution on neutralization." He further states that 78 per cent. of the total nitrogen of linseed is contained in the total protein extracted with alkali.

A great difficulty was encountered in filtering extracts of the meal. This no doubt accounts for the fact that no previous hydrolysis of the proteins of this seed has been attempted, so large a quantity being necessary for a complete hydrolysis. The mucilage in the meal swells up when moistened, and a thick gummy mass results, which resisted filtration by suction. Every artifice was employed, but the only way found possible to obtain a clear filtrate in quantity was by means of a series of large ordinary filters.

Preparations of the proteins were made by two methods.

A. Extraction with 10 per cent. sodium chloride solution.

150 grams of the fat-free meal, 2000 c.c. 10 per cent. sodium chloride solution, and a few drops of toluene to prevent fermentation, were mixed and warmed to 45° C. The mixture was shaken for three hours by mechanical means and allowed to stand all night at 45° C. The liquid was then transferred to a series of ordinary filters, and the clear viscous filtrates obtained during 24 hours, mixed and saturated with ammonium sulphate crystals. The precipitate thus obtained was re-dissolved in 10 per cent. sodium chloride solution and filtered. The filtrate was then submitted to dialysis until entirely free from salts. The globulin found at the bottom of the parchment bag was treated four times with absolute alcohol, and then four times with anhydrous ether to remove water and traces of fat. It was then dried over sulphuric acid. The product thus obtained was crystalline, and the several preparations made contained on the average 17·3 per cent. of nitrogen.

The original intention was to obtain, by this method, sufficient globulin for a complete hydrolysis. The dialysis, however, was found to occupy so much time that it was necessary to resort to a more rapid method. It was also realised at this stage that the globulin did not represent the protein of the linseed as a whole, and that for the purpose in view, a preparation representing as nearly as possible the total protein of linseed was required. Method B was therefore used in making further preparations.

B. Extraction with 0·2 per cent. potassium hydrate solution.

150 grams of the fat-free sifted meal, 2000 c.c. 0·2 per cent. potassium hydrate solution, and a few drops of toluene to delay fermentation, were mixed and warmed to 45° C. The mixture was shaken for three hours, allowed to stand overnight at 45° C., and shaken for one hour next morning. As much as possible was filtered during the succeeding day and night on a series of ordinary filters. The gummy residue was again treated in exactly the same way with fresh potassium hydrate solution, and filtered as before. The mixed filtrates were diluted with five times their volume of distilled water and the protein precipitated by very weak acid, the exact amount required for complete precipitation having been determined by titration. The liquid was siphoned off from the protein which settled in flocks. The precipitate was washed four or five times with large quantities of distilled water and the liquid siphoned off each time. The residue was then allowed to stand overnight in methylated spirit, filtered, and treated three times with absolute alcohol, and finally three times with anhydrous ether. The protein was allowed to dry in the air and was thus obtained as a dry white powder.

One preparation contained

16·28 per cent. nitrogen,
6·26 per cent. water,
0·5 per cent. ash.

Hence the percentage of nitrogen in the dry ash-free substance was 17·45.

Description of the hydrolysis.

365 grams of the protein obtained by Method B, equivalent to 291·1 grams of dry ash and fat-free substance, was suspended in 945 c.c. of hydrochloric acid sp. gr. 1·16. The mixture was warmed on a water bath, and occasionally shaken until the substance passed into solution. It was then boiled under a reflux condenser for ten hours, after which time the biuret reaction had completely disappeared.

Separation of glutaminic acid hydrochloride.

The liquid thus obtained was boiled with animal charcoal, filtered on a Buchner funnel, and the residual charcoal pulverised and thoroughly extracted with hot water. The filtrate and washings were evaporated in vacuo to 650 c.c., thoroughly saturated with dry hydrochloric acid gas and allowed to stand at 0° C. to freeze out the glutaminic acid

hydrochloride. After three days only a very few crystals appeared. It was then placed in a mixture of ice and salt for eight hours and again kept at 0° C. On the fifth day a crystalline deposit a quarter of an inch in thickness was found. The flask was again cooled for nine hours in ice and salt, and then kept at 0° C., when the crystalline deposit continued to increase until the seventh day, after which time no further crystals could be obtained. These details are mentioned because the crystallisation of the glutaminic acid hydrochloride is sometimes a great difficulty. The ice-cold mixture was then diluted with an equal volume of ice-cold absolute alcohol, the crystals filtered off on a Buchner funnel and washed with ice-cold alcohol previously saturated with hydrochloric acid gas. The crystalline hydrochloride was then dissolved in water, boiled with animal charcoal and filtered. The colourless filtrate was then evaporated to crystallisation.

41·36 grams of glutaminic acid hydrochloride were thus obtained.

0·2308 grams of the preparation, by Kjeldahl's method, required 12·6 c.c. N/10 acid. The substance therefore contained 7·64 per cent. nitrogen. Glutaminic acid hydrochloride contains 7·62 per cent.

Esterification of the amino-acids.

The liquid from which the glutaminic acid had been removed was evaporated in vacuo below 50° C. to a thick syrup. This was dissolved in 1000 c.c. absolute alcohol, saturated with dry gaseous hydrochloric acid and warmed on the water bath for half an hour, by which means most of the amino-acids were esterified. The liquid was then again concentrated to a thick syrup in vacuo below 50° C. to remove the water produced in the esterification. The syrup was again dissolved in 1000 c.c. absolute alcohol, saturated with dry gaseous hydrochloric acid, and warmed on a water bath. The whole operation was again repeated, when it was judged that complete esterification had taken place. The syrup was now dissolved in 600 c.c. absolute alcohol, again saturated with dry gaseous hydrochloric acid, seeded with a crystal of glycine ester hydrochloride and allowed to stand 48 hours at 0° C. There was deposited a very small quantity of a crystalline substance, which showed no increase during a further 48 hours at 0° C. It was filtered off, washed with absolute alcohol which had been previously saturated with dry gaseous hydrochloric acid, dried, and weighed 0·46 grams. The crystals appeared under the microscope to be glutaminic acid hydrochloride. No glycine ester hydrochloride could

be isolated at this stage. The liquid was again evaporated in vacuo to a syrup, and the esters extracted by the method of Levene[1].

Extraction of the esters by Levene's method.

The syrup was introduced into a suitable vessel and the little remaining in the flask washed out with a very small quantity of water. It was allowed to stand in a freezing mixture of ice and salt, and very finely ground crystalline baryta added, with continual stirring, until an alkaline reaction appeared. The vessel used was made of metal coated with enamel, and the stirring was done with a strong wooden spatula. The rapid conduction of heat by the metal to the freezing mixture facilitated the effect of the latter, and the strength of the vessel allowed the mixture to be stirred with considerable vigour. Anhydrous baryta was then added in portions of 10—15 grams at a time, alternately with repeated additions of anhydrous ether, the ethereal layers being poured off from time to time. The addition of anhydrous baryta was continued until the substance at the bottom became granular, and the ethereal layer quite clear. During the whole operation continual stirring and mixing of the ingredients ensured proper cooling by the freezing mixture, and proper extraction of the esters by the ether. The ethereal solution of the esters, measuring about three litres, was shaken up with potassium carbonate and allowed to stand a long time over anhydrous sodium sulphate to remove traces of water.

According to Levene, the esters are only partially removed by one or even two extractions. The residue left in the vessel was therefore very thoroughly extracted with water, and the barium quantitatively removed from the solution by means of sulphuric acid. The solution was concentrated to a syrup in vacuo below 50° C. and the remaining amino acids again esterified by dissolving in absolute alcohol and saturating with dry gaseous hydrochloric acid. Two more similar operations were carried out to insure complete esterification by getting rid of the water formed. The resulting syrup was then subjected to a further application of Levene's method of extraction exactly as before. After a third exactly similar treatment and a third ethereal extraction, it was judged that as much as possible of the esters had been obtained.

Levene's method is found to give a larger yield of esters and is very much easier to carry out than the older methods[2]. The sparing solubility

[1] *Journ. Biological Chemistry*, Vol. VI. Sep. 1909, No. 5.

[2] *Chemical constitution of Proteins*, Plimmer, p. 9.

of the baryta prevents destruction of the esters at so low a temperature, and therefore possesses an advantage over the caustic soda used in the older methods. At the same time, anhydrous baryta effectually salts out the esters and removes the water.

Fractional distillation of the esters by Fischer's method.

The ether was evaporated at a temperature below 40° C. from the combined ethereal solutions which had been left standing in the ice chest over sodium sulphate for some days. The liquid was then transferred to a distilling flask and the remaining ether distilled off in vacuo. The ethereal distillates were preserved and afterwards worked up to be examined for glycine.

The esters were then fractionally distilled by the aid of an apparatus exactly similar to that described by Fischer[1]. Five fractions were obtained, the first two at a pressure of from 10 to 12 mm., obtained by a very efficient water pump, and the last three at 0·5 to 0·8 mm. by absorption of the remaining gases with freshly prepared Dewar's cocoa-nut fibre charcoal surrounded by liquid air.

Particulars of the fractions obtained.

	Temperature of bath	Pressure	Weight of fraction
Fraction 1.....	up to 60° C.	12 mm.	39·2 grams
,, 2.....	60° to 91° C.	10 mm.	3·7 ,,
,, 3.....	91° to 102° C.	0·8 mm.	69·7 ,,
,, 4.....	102° to 131° C.	0·7 mm.	50·3 ,,
,, 5.....	131° to 172° C.	0·5 mm.	11·7 ,,
		Total	174·6 grams

During the course of the distillation, as the temperature of the bath was gradually raised, it was noticed that the rate of distillation was not uniform. Distillation practically ceased at 85° to 91° C. The pressure was then lowered, when distillation became very rapid between 94° and 99° C. Between 99° and 102° C. scarcely a drop came over. Scarcely any residue was left in the distilling flask.

Extraction of phenyl-alanine ester by means of ether from Fractions 4 and 5.

Five times the volume of water was added to each of these fractions in separating funnels. In the case of Fraction 5, a fair quantity of the

[1] Fischer and Harries, Ber. 1902, 35. 2158–2162.

phenyl-alanine ester separated as an oil. The mixtures were shaken, and an equal volume of ether added to each, again shaken for a few minutes, and allowed to stand. The ethereal layers were each washed three times with water and the washings returned to their respective fractions. The combined ethereal solutions were then entirely freed from ether by evaporation, and the ester weighed.

Weight of phenyl-alanine ester = 29·82 gm.

Strong hydrochloric acid was added to the ester and evaporated down. This was done twice more. A crop of crystals was then removed and the mother liquor reduced in bulk by evaporation. A second small crop was then filtered off. A third reduction of bulk of the mother liquor resulted in a small quantity of entirely different crystals. Dried at 100° C. these weighed 0·57 gm. and were small well-formed nodules exactly resembling those of leucine. The first two crops were therefore taken to represent the whole of the phenyl-alanine. The hydrochloride was re-crystallised from hot strong hydrochloric acid, dried and weighed.

Weight of phenyl-alanine hydrochloride, 14·73 gm. equal to 12·06 gm. of free phenyl-alanine corresponding to 4·14 per cent. of phenyl-alanine in the protein.

0·3128 gm. of the hydrochloride taken out from the whole well-mixed bulk of the re-crystallised substance, after thorough drying at 100° C. gave, by Kjeldahl's method, ammonia neutralising 15·75 c.c. N/10 acid, equal to 7·05 per cent. nitrogen. Phenyl-alanine hydrochloride contains 6·95 per cent. nitrogen.

Separation of glycine and alanine.

The ethereal distillate obtained by evaporation at 40° C. from the ethereal solution of the esters before fractionation was shaken three times with a little dilute hydrochloric acid, and the ether distilled. The residual liquid in the distilling flask was added to the acid layers, evaporated to dryness, dried and weighed. 0·31 gm. was obtained, which was added to Fraction 1.

Fraction 1. Temperature of bath up to 60° C., pressure 12 mm. To this fraction, which should contain chiefly glycine and alanine, strong hydrochloric acid was added, and the solution evaporated to complete dryness on the water bath in a weighed dish and dried. This treatment decomposes the esters, and gives hydrochlorides.

Weight of hydrochlorides 3·18 gm.

To this was added 0·31 gm. obtained from the ether distillates, making 3·49 gm. in all. This was dissolved in absolute alcohol and evaporated to about 30 c.c., saturated with dry gaseous hydrochloric acid, and seeded with a minute crystal of glycine ester hydrochloride. It was allowed to stand at 0° C. for about a month, and at odd times in a mixture of ice and salt. A very small quantity separated, which was filtered off, dried, and weighed 0·016 gm., M.P. 147° C. Glycine ester hydrochloride melts at 144° C.

Strong hydrochloric acid was next added to the liquid, which was then evaporated to small bulk to convert the ester hydrochlorides into acid hydrochlorides. Most of the hydrochloric acid was then removed by boiling with lead oxide and filtering off the lead chloride after cooling. The rest of the lead was removed from the filtrate by sulphuretted hydrogen. This was boiled off and sufficient silver sulphate added to remove the small quantity of hydrochloric acid remaining. Silver was removed from the filtrate by sulphuretted hydrogen, which was boiled off and the sulphate then precipitated quantitatively with baryta. The liquid containing the free amino-acids was then evaporated to crystallising point and allowed to crystallise. The whole of the solution was finally evaporated to complete dryness, pulverised and dried to constant weight at 100° C. The substance melted sharply at 298° C.

The melting point of alanine is 297° C.

Weight of alanine thus obtained = 2·39 gm.

The following results were obtained on combustion :—

0·2233 gm. gave 0·3433 gm. CO_2 and 0·160 gm. H_2O = 41·9 per cent. carbon and 7·96 per cent. hydrogen.

Alanine contains 40·45 per cent. carbon and 7·86 per cent. hydrogen.

Fraction 2. Weight of esters 3·67 gm., temperature of the bath 60—91° C., pressure 10 mm.

The esters were converted into the amino-acids by boiling with five to six times their volume of water under a reflux condenser for eight hours. The liquid was then evaporated to dryness in a weighed dish on the water bath, and the residue pulverised and dried at 100° C. The melting point of the mixed amino-acids was 251° C.

The proline was extracted from the substance by boiling with absolute alcohol, cooling, and allowing to stand a long time. Weight of dry extract = 0·254 gm. This was re-dissolved in absolute alcohol,

cooled and filtered, dried and weighed again = 0·18 gm. The same process was again repeated and there was finally obtained 0·16 gm. proline, which was added to that extracted from fraction 3.

A combustion of the remaining substance after thorough drying at 100° C. was then carried out.

0·1973 gm. gave 0·1395 gm. H_2O and 0·3198 gm. CO_2.

7·86 per cent. hydrogen and 44·21 per cent. carbon.

The substance was then dissolved in water and subjected to fractional crystallisation, and 0·43 gm. of well-formed nodular crystals of leucine were separated. These were re-crystallised and, after thorough drying, found to melt sharply at 283° C.

There was further separated from this fraction 0·6 gm. resembling alanine and 0·7 gm. of a characteristic substance crystallising in well-formed needle aggregates. The latter turned brown at 240° C. and melted sharply at 260° C. It was preserved for further investigation.

Fraction 3. Temperature of bath 91—102° C., pressure 0·8 mm., weight of esters 69·66 gm.

The esters were hydrolysed by boiling with 400 c.c. of water under a reflux condenser, until the liquid was no longer alkaline to litmus. About 12 hours was found necessary. After several days' standing, about 10 gm. of very large crystals were found attached to the bottom in clusters. No characteristic leucine nodules could be seen. The whole fraction was evaporated to dryness, the amino-acids being removed from time to time to prevent loss by spitting.

The total dried solid substance weighed 55·19 gm.

Extraction of proline.

The mixed amino-acids were boiled with absolute alcohol, cooled, allowed to stand overnight, filtered and washed with alcohol. The residue was extracted thrice more in the same manner. The combined alcoholic extracts were evaporated to dryness, and this residue freed from small quantities of the other amino-acids by repeated extraction and evaporation. The residue finally dissolved quite readily in absolute alcohol, and nothing separated when cooled and allowed to stand a long time. The brownish residue dried in the steam oven weighed 8·57 gm. To this 0·16 gm. extracted from fraction 2 in a similar manner was added, making in all 8·73 gm. of proline. This was dissolved in water and converted into its copper salt by boiling with copper hydroxide on

a water-bath for three quarters of an hour. The filtrate was evaporated to dryness, and the mixed copper salts of laevo- and racemic proline dried at 100° C. weighed 10·31 gm.

0·1217 gm. gave 0·0303 gm. CuO = 19·9 per cent. Cu.

Copper proline—$C_{10}H_{16}O_4N_2Cu$—contains 21·81 per cent. Cu.

The residue was treated with water when it was found that a small quantity of a lighter coloured copper salt did not dissolve even when slightly warmed. This was filtered off, boiled with more copper hydroxide, filtered hot and concentrated to crystallisation. 0·546 gm. of a copper salt was thus obtained, pink-purple in colour when dried at 100° C., but regaining its light-blue colour on exposure to air.

0·1359 gm. gave 0·0336 gm. CuO = 19·45 per cent. Cu.

Copper leucine contains 19·64 per cent. Cu.

It was therefore concluded that this substance was the copper salt of leucine, corresponding to 0·44 gm. leucine.

The solution of the mixed proline copper salts was boiled with more copper hydroxide, and the filtrate evaporated to dryness, powdered and dried at 100° C. The copper laevo-proline was separated from the racemic salt by boiling with absolute alcohol, in which the latter remained undissolved. The cold alcoholic solution of the laevo-proline copper salt deposited a very small quantity of well-formed needles on standing. According to Osborne[1], the copper salt of laevo-proline is amorphous.

The laevo-proline copper, dried to constant weight at 135° C., weighed 8·15 gm.

0·2351 gm. gave 0·0521 gm. CuO = 20·33 per cent.

Calculated for $C_{10}H_{16}O_4N_2Cu$ = 21·81 per cent of Cu.

The writer is unable to account for the 1·5 per cent. deficiency in content of copper. The original proline extracted with absolute alcohol from the other amino-acids had the characteristic brown colour, and readily, as well as completely, dissolved in a small volume of absolute alcohol, no further deposit being produced on long standing. The content of copper in fractions obtained on evaporation of the alcoholic solution of the laevo-copper salt was estimated after drying to constant weight at 135° C., but 20·45 was the highest percentage obtained in any fraction.

The undissolved racemic copper salt dried at 100° C. weighed 1·22 gm. On crystallisation from water, a deposit was obtained which had not the appearance of the racemic salt. Many attempts were

[1] *Amer. Journ. Physiol.* Vol. xx. Jan. 1, 1908, No. iv.

made to obtain the characteristic plates, but only an unweighable quantity separated.

Separation of leucine and valine.

The residual amino-acids of fraction 3 from which the proline had been extracted were dried at 100° C., and weighed 45·73 gm. The mixture melted sharply at 274° C., and on combustion gave the following results:

A. 0·2447 gm. gave 0·4627 gm. CO_2, and 0·2046 gm. $H_2O = 51·57$ per cent. carbon, 9·29 per cent. hydrogen.

B. 0·2246 gm. gave 0·4228 gm. CO_2, and 0·1894 gm. $H_2O = 51·34$ per cent. carbon, and 9·37 per cent. hydrogen.

1·1891 gm. dried to constant weight at 100° C. lost 0·0022 gm. = 0·185 per cent. water.

Valine contains 51·24 per cent. carbon, 9·4 per cent. hydrogen.

Leucine 54·92 per cent. carbon, 9·92 per cent. hydrogen.

The specific rotation of the mixed acids in 20 per cent. hydrochloric acid was then taken. For this purpose 0·998 gm. was dissolved in 25 c.c. of the acid at 20° C., and the solution introduced into a 100 mm. tube. The reading obtained was + 0°·92, and the specific rotation was calculated as follows:

$$\left[\alpha\right]_D^{20^\circ} = \frac{\cdot 92 \times 25}{\cdot 998 \times 1} = +23^\circ\cdot 04.$$

For d-valine $\left[\alpha\right]_D^{20^\circ} = +27^\circ\cdot 9$[1], for leucine + 15°·6[2], and for d-iso-leucine + 37°·4[2]. It is evident that the rotation gives no definite information as to the composition of the sample of mixed amino-acids, since both leucine and iso-leucine may be present together with the valine, and small quantities may have become racemised during the evaporations and drying at 100° C.

The nitrogen was determined in the mixed amino-acids by Kjeldahl's method.

A. 0·2457 gm. gave ammonia neutralising 20·5 c.c. N/10 acid, equal to 11·68 per cent. nitrogen.

B. 0·2668 gm. gave ammonia neutralising 22·2 c.c. N/10 acid, equal to 11·65 per cent. nitrogen.

[1] Schultze and Winterstein, *Zeitschr. f. physiol. chem.* 35. 299 (1902).

[2] Levene, *loc. cit.*

Mean result = 11·66 per cent. nitrogen, equal to 11·68 per cent. nitrogen in the moisture-free substance.

Valine contains 11·97 per cent. nitrogen.

Leucine contains 10·68 per cent. nitrogen.

The figures indicate that the 45·73 gm. consisted principally of valine. To further verify this, the method of Levene[1] and van Slyke for separation of leucine from valine was applied. It was decided to work on the figures given by Combustion A, which, after correction for the trace of water present in the substance, indicate 51·66 per cent. of carbon in the mixed amino-acids.

From this figure the relative proportions of leucine and valine in the mixture were calculated as follows:

$$\frac{\text{Percentage of carbon in mixture} - \text{percentage of carbon in valine}}{\text{Percentage of carbon in leucine} - \text{percentage of carbon in valine}} \times 100$$

$$= \frac{51{\cdot}66 - 51{\cdot}24}{51{\cdot}92 - 51{\cdot}24} \times 100 = 11{\cdot}58 \text{ per cent. of leucine isomers in the mixture.}$$

To 38 gm. of the mixed amino-acids, seven times its weight, *i.e.* 266 c.c. of water was added, and heated to boiling. To the hot liquid 57 c.c. ammonia (s.g. 0·9)—*i.e.* 1·5 c.c. for each gram of mixed acids taken—was added, and the mixture shaken to dissolve all the acids. For every gram of leucine calculated to be present, 4 c.c. of a solution of lead acetate containing 1·1 gm. molecules per litre was added, *i.e.* 17·6 c.c. This solution was added very slowly with constant vigorous stirring to prevent local precipitation of the lead salt of valine. The liquid was then chilled under the tap, and allowed to stand. The lead salts of the leucine isomers were then filtered off on a Buchner funnel, washed very thoroughly with 90 per cent. alcohol, and finally with ether, and allowed to stand in a vacuum desiccator over sulphuric acid for 48 hours. According to Levene "the presence of 3 per cent. or more, of valine, makes the precipitation of the leucines practically quantitative, the valine exerting a salting out effect."

An estimation of lead in the leucine lead salt was made. 0·2429 gm. gave 0·1595 gm. $PbSO_4$, which corresponds to 44·87 per cent. of lead. $Pb(C_6H_{12}O_2N)_2$ contains 44·34 per cent. lead.

The lead was removed from the remainder of the precipitate by sulphuretted hydrogen, and the leucine crystallised from water in characteristic nodules.

[1] *Journal of Biological Chemistry*, Vol. VI. 1909, p. 391.

A calculation was now made from the nitrogen content of the mixed amino-acids as follows:

$$100 \times \frac{\text{per cent. of nitrogen in mixture} - \text{per cent. of nitrogen in leucine}}{\text{per cent. nitrogen in valine} - \text{per cent. nitrogen in leucine}}$$

$$100 \times \frac{11{\cdot}69 - 10{\cdot}68}{1{\cdot}28} = 78{\cdot}6 \text{ per cent. valine in mixture.}$$

A second fraction of lead salt was therefore precipitated as before, the two fractions corresponding with 21·4 (100 − 78·6) per cent. of leucine. The lead salt thus precipitated was purified as before, and the lead estimated as sulphate.

0·2683 gm. gave 0·1825 gm. $PbSO_4$ = 46·45 per cent. of lead. Lead valine contains 47·18 per cent. of lead.

Unfortunately the mother liquor from which the first lead salt had been precipitated, was allowed to stand about 48 hours whilst the precipitate was being purified, dried and analysed. A very small quantity of white substance was deposited during this time, and when too late it was realised that this should have been filtered off before precipitating the second lead fraction. As the liquid contained ammonia and also a small quantity of lead in solution, the deposit was probably for the most part basic. The lead content was therefore considered an unreliable criterion, and, in the writer's opinion, the less the filtration of any of these lead precipitates is delayed, the more reliable the lead content becomes. The percentage of lead in the first lead precipitate is probably also a little too high on this account.

The lead was removed from the remainder of the second lead fraction by means of sulphuretted hydrogen, the solution evaporated to dryness, and traces of acetic acid and ammonium acetate removed by washing with a mixture of absolute alcohol and ether as directed by Levene. The content of nitrogen in the free amino-acid was then determined by Kjeldahl's method.

A. 0·2504 gm. substance evolved ammonia neutralising 19·2 c.c. N/10 acid, equal to 10·73 per cent. nitrogen.

B. 0·2473 gm. substance evolved ammonia neutralising 19·3 c.c. N/10 acid, equal to 10·88 per cent. nitrogen.

Percentage of nitrogen in leucine = 10·68.

From the nitrogen determination which is much more reliable than that of the lead, it was concluded that this fraction consists chiefly of leucine.

The filtrate from the second lead fraction was freed from lead with sulphuretted hydrogen evaporated to dryness, washed with a mixture of alcohol and ether as before, and dried. The nitrogen was then estimated by Kjeldahl's method.

0·2307 gm. gave ammonia neutralising 19·45 c.c. N/10 acid equal to 11·80 per cent. nitrogen. Valine contains 11·97 per cent. nitrogen.

A combustion was also carried out, and the following results obtained:

0·2211 gm. gave 0·4133 gm. CO_2, and 0·1861 gm. $H_2O = 50·99$ per cent. carbon and 9·36 per cent. hydrogen. Valine contains 51·24 per cent. carbon and 9·4 per cent. hydrogen.

It has been shown above that of the 45·73 gm. of mixed amino-acids, just under 21·4 per cent. consists of leucine. Probably it will not be far from the mark to conclude that 20 per cent. of the mixture consisted of leucine. This corresponds to 9 gm. leucine and 37 gm. of valine, equivalent to 12·7 per cent. valine in the protein.

Separation of aspartic acid from Fractions 4 *and* 5.

Fraction 4. Temperature of bath 102°—131° C., pressure 0·7 mm. The esters were hydrolysed by boiling with five times their volume of baryta water for three hours. The mixture was allowed to stand several days. A small quantity of barium aspartate separated out, which was filtered off.

Fraction 5. Temperature of the bath 131°—172° C., pressure 0·5 mm. These esters were hydrolysed in exactly the same way as those of fraction 4. A larger amount of barium aspartate separated however. This was filtered off, and added to that obtained from fraction 4.

The barium was removed quantitatively by means of sulphuric acid, and the filtrate evaporated to dryness in a weighed dish. The residue was pulverised, dried at 100° C., and weighed 3·55 gm.

0·1908 gm. of the substance by Kjeldahl's method gave ammonia neutralising 13·95 c.c. N/10 acid. The preparation therefore contained 10·24 per cent. of nitrogen. Aspartic acid contains 10·52 per cent. of nitrogen.

The filtrates from the barium aspartate precipitates of fractions 4 and 5 were then combined, and worked up together. The barium was removed quantitatively by means of sulphuric acid, and the liquid evaporated to 130 c.c. It was saturated with dry gaseous hydrochloric

acid in a freezing mixture, in which it was allowed to stand all night. It was then placed in the ice-chest for several days. A little glutaminic acid hydrochloride separated, which failed to increase on further standing. On the top of the liquid, however, a small quantity of large brownish crystals separated, which were removed and preserved. A few more crystals of a similar nature were removed at a later stage, making in all about 0·4 gm.

The small quantity of glutaminic acid hydrochloride was filtered, dried, and weighed 0·26 gm.

The filtrate was evaporated to 80 c.c., again saturated with dry hydrochloric acid gas, and allowed to stand a long time at 0° C. A white substance separated which was not glutaminic acid hydrochloride, and after adding water which readily dissolved the white substance, a few more of the brownish crystals mentioned above were picked out.

Most of the hydrochloric acid was then removed from the liquor by evaporation, and the remainder completely eliminated by means of lead oxide and silver sulphate, as described in the case of fraction 1. The resulting liquid containing the free acids was evaporated to small bulk, and boiled with excess of pure copper hydroxide. On cooling the liquid, which measured about 200 c.c., light-blue needle aggregates were deposited. The weight of copper aspartate thus obtained = 1·61 gm., equal to 1·24 gm. of aspartic acid.

The content of copper in the air-dried copper aspartate was then estimated.

0·2197 gm. gave 0·0578 gm. CuO = 21·04 per cent. copper. The remainder was dissolved in water, re-crystallised, and the very characteristic crystals exposed to the air until they could be ground to a very fine powder. After a further long period of exposure to the air, the copper was again estimated.

0·2022 gm. gave 0·536 gm. CuO = 21·17 per cent. of copper.

Copper aspartate $C_4H_5NO_4Cu\ 4\frac{1}{2}\ H_2O$ contains 23·07 per cent. of copper.

The crystals separated from a large bulk of liquid and had the characteristic appearance of copper aspartate, and the above figures suggest that the molecule contains more water of crystallisation than is generally supposed to be present.

The filtrate was reduced much further in bulk, and two further quantities of crystals quite unlike the previous crop were removed. These were dried at 100° C., and weighed 1·37 and 0·56 gm. The

copper was removed from these by sulphuretted hydrogen, and 1·13 gm. of a substance crystallising in nodules resembling leucine was obtained.

The mother liquor from which no further copper salts would crystallise, was freed from copper by sulphuretted hydrogen. The sulphuretted hydrogen was removed by boiling, and the liquid allowed to evaporate in a vacuum desiccator over sulphuric acid. A very small quantity of substance resembling serine was discovered in the gummy residue, but was too small in amount to be easily separated. The β-naphthalene-sulpho derivative was then made by the method of Fischer[1] and Bergell, the whole of the gummy residue being used for this purpose. The mixture of the ethereal solution of the β-naphthalene-sulpho chloride with the solution of the amino-acid in normal soda was found to be slightly alkaline after the first period of 1½ hours in the mechanical shaker, and further addition of soda was subsequently found unnecessary. The solution was separated from the ethereal layer, filtered, and dry hydrochloric acid gas passed in. After a short time a turbidity appeared, and on further addition of the hydrochloric acid gas, a very small quantity of white substance was deposited. This was re-dissolved in the smallest possible quantity of very dilute soda, filtered, and dry hydrochloric acid gas again passed in. The turbidity again appeared at first, and the passing of the hydrochloric acid gas was discontinued before the liquid became sufficiently saturated for sodium chloride also to separate. The deposit thus obtained was examined under the microscope, and was found to consist of flat lumpy crystals made up of clusters of needles. These were re-crystallised from hot alcohol, and the β-naphthalene-sulpho serine with no water of crystallisation thus prepared, consisted of tiny needles for the most part in star-shaped clusters, but the quantity was too small to collect and weigh. This anhydrous substance melted at 219·5° C.

Tyrosine.

A quantity of protein extracted by Method B, equal to 35·84 gm. of dry ash-free protein, was boiled for 24 hours with a mixture of 120 gm. concentrated sulphuric acid, and 240 c.c. of water. The cold liquid was poured into the calculated quantity of baryta suspended in 2000 c.c. water, and well shaken. The barium sulphate was filtered off, and thoroughly extracted with boiling water. The combined filtrate and washings were found to be slightly alkaline. Carbon dioxide was

[1] *Ber. d. deutschen chemischen Gesellschaft*, 1902, 34. 3779,

passed in, and the liquid evaporated to small bulk. This evaporation in an open dish with the barium carbonate served to drive off all ammonia. The barium carbonate was filtered off and washed, and the little remaining barium exactly removed from the liquid by sulphuric acid.

The liquid was evaporated on the water bath until a substance commenced to crystallise. After allowing to stand at 0° C. for 24 hours, it was filtered and dried as far as possible by suction. The filtrate was reduced a little further in bulk, and again allowed to stand at 0° C. and filtered. In a similar way four small portions were removed, but the mother liquor was still found to give a slight red colour on boiling with Millon's reagent. The estimation of arginine, histidine and lysine was, however, proceeded with, and a further attempt made to isolate more tyrosine after removal of the arginine and histidine with silver sulphate and baryta, and the lysine with phosphotungstic acid.

Histidine, Arginine and Lysine.

These were estimated by the method of Kossel and Patten[1], modified to some extent as suggested by Osborne, Leavenworth and Brautlecht[2].

The filtrate from which most of the tyrosine had been removed was made up to 3000 c.c. in a five-litre flask, and heated on a water bath. Finely powdered silver sulphate was added until the solution contained sufficient to give a yellowish-brown precipitate on testing a drop with baryta water. The liquid was cooled to 40° C., and saturated with finely powdered baryta. The silver salts of arginine and histidine were filtered off, and stirred up with baryta water, and again filtered and washed with baryta water. The washings were added to the filtrate, and the liquid was preserved for the estimation of lysine.

The precipitate of mixed Silver Salts of Histidine and Arginine.

This was suspended in a litre of water acidified with sulphuric acid, and the silver precipitated with sulphuretted hydrogen. The silver sulphide was filtered off, and washed with sulphuretted hydrogen water. The residue was again suspended in water, and the treatment with sulphuretted hydrogen repeated. The two filtrates were united, the sulphuretted hydrogen removed by evaporation to about 250 c.c., the slight sediment filtered, and the liquid made up to 500 c.c.

[1] *Zeitschr. für physiol. Chem.* 1903, XXXVIII. 39.

[2] *American Journal of Physiology*, 1908, Vol. XXIII. No. 3.

The nitrogen in the liquid was estimated by Kjeldahl's method to be 1·155 gm. N in the 500 c.c.

The silver sulphide residue was once more thoroughly extracted by boiling with water three times, filtering and washing with hot water. The combined washings were evaporated and made up to 200 c.c., and the nitrogen estimated by Kjeldahl's method to be 0·013 gm. in the 200 c.c.

The remainder of this liquid was added to the previous filtrate. The total nitrogen present as histidine and arginine therefore amounted to 1·168 gm.

The liquid was then evaporated to 250 c.c., and the sulphuric acid estimated by titration. Sulphuric acid was added until the solution contained 5 per cent., and the histidine precipitated with mercuric sulphate, taking care not to add too large an excess. After standing 24 hours, the mercury-histidine was filtered off, and washed thoroughly with 5 per cent. sulphuric acid. By this method most of the histidine is precipitated as mercury salt, but a little remains in solution with the arginine.

Treatment of Filtrate to separate the remaining Histidine.

This was freed from mercury by sulphuretted hydrogen, and from sulphuric acid by neutralising to litmus with baryta, and adding barium nitrate as long as a precipitate was formed. Silver nitrate was then added to the filtered liquid until a drop added to baryta water produced a brownish precipitate. The histidine-silver was precipitated by adding barium hydroxide until the solution was neutral to litmus, and an attempt made to make the precipitation complete as directed by Osborne and his colleagues in the paper previously quoted. He there states that complete precipitation of the remaining histidine-silver is brought about by the addition of 5 c.c. of saturated baryta solution, and subsequent additions of 2 c.c. portions until 10 c.c. of the liquid filtered clear gives no further precipitate on adding a drop of the baryta solution. The writer, however, was quite unable to arrive at a point when no further precipitate could be obtained on addition of baryta. The successive additions of baryta in 2 c.c. portions after the manner stated was persevered with until the liquid was strongly alkaline, and the bulk of the precipitate obtained left no possible doubt that a large quantity of arginine-silver had come down, as well as the histidine-silver. The method for separating the

remaining histidine-silver was therefore abandoned as being quite unworkable.

The barium and silver were removed by sulphuric acid and sulphuretted hydrogen respectively, and the whole process repeated up to the point of neutrality to litmus on the addition of baryta water after adding the silver nitrate solution. Further small quantities of saturated baryta water were then added from a burette, until the liquid ceased to give the following test: A drop of the liquid was allowed to fall through a small filter on to a white glazed tile. A drop of ammoniacal silver solution was placed alongside, and the two drops made to run together. A precipitate was formed which was easily soluble in excess of ammonia as long as any histidine remained in solution. Complete precipitation of histidine was brought about after the addition of very little extra baryta solution. The small precipitate was filtered off, stirred up with water, again filtered off by suction, and washed thoroughly with water. The small quantity of histidine-silver thus recovered was added to the histidine-silver subsequently obtained from the mercury precipitate. The filtrate contained the arginine only.

Filtrate containing the Arginine only.

This liquid was saturated with baryta, the precipitate stirred up with saturated baryta solution, and again filtered and washed with saturated baryta solution until it was quite free from nitrate. It was then suspended in water containing sulphuric acid, and decomposed with sulphuretted hydrogen. The filtrate from the well-washed silver sulphide was evaporated down, made up to 250 c.c., and the nitrogen present as arginine determined by Kjeldahl's method. After making allowance for the portions taken out for previous Kjeldahl estimations, there resulted 0·699 gm. nitrogen as arginine, equivalent to 2·173 gm.

$$\text{arginine} = \frac{2{\cdot}173 \times 100}{35{\cdot}84} = 6{\cdot}06 \text{ per cent. in the original protein.}$$

The remainder of the liquid was freed from sulphuric acid by baryta, the excess of which was removed by carbon dioxide. The liquid was then neutralised with nitric acid and evaporated. A white crystalline mass of arginine nitrate was thus obtained.

Histidine in the Mercury-Histidine precipitate.

The histidine in this precipitate after removal of the mercury with sulphuretted hydrogen, was converted into the silver compound as follows: The mercury sulphide was filtered off, stirred up with hot

water, again filtered and washed. The filtrate was evaporated to 150 c.c., neutralised to litmus with baryta water, and barium nitrate added till no further precipitate was obtained. To the filtrate from this, silver nitrate was added until a brown precipitate was produced on addition of a drop of baryta water. Cold saturated baryta water was then added until no further precipitate was produced on testing a drop with ammoniacal silver solution. The histidine-silver thus precipitated was added to the other small amount separated from the arginine.

The combined precipitates were suspended in water acidified with sulphuric acid, and the silver removed by sulphuretted hydrogen. The filtrate was made up to 250 c.c., and the nitrogen estimated by Kjeldahl's method. After making allowance for portions taken out for previous Kjeldahl estimations, 0·161 gm. nitrogen as histidine was found.

0·161 gm. nitrogen is equivalent to 0·593 gm. histidine ($C_6H_9N_3O_2$). $0{\cdot}593 \times \frac{100}{35{\cdot}84} = 1{\cdot}66$ per cent. in the original protein. The histidine hydrochloride was obtained as a white crystalline substance as follows:

Baryta was added until alkaline, and the barium sulphate filtered off. Carbon dioxide was then passed in, and the liquid evaporated to dryness. The residue was extracted with boiling water, and the barium carbonate removed. The filtrate was again evaporated to dryness, extracted and filtered as before to remove all traces of barium carbonate. On evaporating down with hydrochloric acid, a small quantity of crystalline histidine hydrochloride was obtained.

Lysine.

This was obtained from the filtrate from the precipitate of histidine and arginine. The liquid was made acid with sulphuric acid, and the silver removed with sulphuretted hydrogen. The filtrate was evaporated and made up to 500 c.c., and sulphuric acid added until the liquid contained 5 per cent. The lysine was then precipitated with phosphotungstic acid. The latter was added until no further precipitate was obtained. The lysine phosphotungstate was stirred up with a solution of 5 per cent. phosphotungstic acid in 5 per cent. sulphuric acid, filtered and washed, and the operation twice repeated.

The combined filtrate and washings containing the mono-amino-acids and a little remaining tyrosine were preserved and subsequently treated.

The lysine phosphotungstate was suspended in 600 c.c. of water, and baryta added in small quantities at a time to the solution at 40° C. with continuous shaking until it was alkaline. The barium phosphotungstate was filtered off and carbon dioxide passed into the filtrate. The liquid was then evaporated nearly to dryness, extracted with water, and the barium carbonate filtered off. The liquid was again evaporated down to as small a bulk as possible, and an alcoholic solution of picric acid added until a precipitate ceased to be formed, taking great care not to add excess, which would have re-dissolved the lysine picrate. After standing twelve hours it was filtered off and washed with a very small quantity of absolute alcohol. The precipitate was then dissolved in boiling water, and the solution evaporated. The beautiful crystals were removed in three portions on weighed filters, and the mother liquor could not be induced to deposit a further weighable quantity. Total weight of $C_6H_{14}N_2O_2C_6H_2(NO_2)_3OH$ obtained = 1·10 gm. This is equivalent to 0·428 gm. lysine. $0{\cdot}428 \times \frac{100}{35{\cdot}84} = 1{\cdot}19$ per cent. lysine in the original protein.

The percentage of nitrogen in the picrate was estimated by Kjeldahl's method. 0·139 gm. gave NH_3 neutralising 18·35 c.c. N/10 acid equal to 18·49 per cent. nitrogen. Lysine picrate contains 18·67 per cent. of nitrogen.

Tyrosine.

The phosphotungstic acid and sulphuric acid in the filtrate from the lysine phosphotungstate were exactly removed with baryta. The neutral filtrate was evaporated until a white substance commenced to crystallise, when it was allowed to stand at 0° C. for some time. Four small portions were removed altogether in a similar manner, when the mother liquor was found to give only the very faintest possible red tinge on boiling with Millon's reagent. These fractions were added to those obtained before the removal of the diamino-acids. The combined fractions were dissolved in water and decolourised by boiling with purified animal charcoal. The liquid was then filtered by suction, the charcoal pulverised and boiled two or three times with water. A little dilute ammonia was added to the combined filtrate and washings, and the clear colourless liquid evaporated to crystallising point. A little more dilute ammonia was then added, and the liquid allowed to stand a long time. The substance was examined under the microscope, and was found to consist entirely of well-formed needles.

The tyrosine thus obtained dried at 100° C. weighed 0·233 gm. = 0·65 per cent. in the protein.

0·1078 gm. of the substance by Kjeldahl's method gave NH_3 neutralising 14·0 c.c. N/10 acid equal to 7·79 per cent. of nitrogen. Tyrosine contains 7·73 per cent. of nitrogen.

Nothing resembling tyrosine was further obtained from the mother liquor after much time spent, and only a faint red colour resulted on boiling the liquid with Millon's reagent.

Ammonia.

A quantity of protein prepared by Method B, equal to 1·21 gm. moisture, ash and fat-free substance, was hydrolysed by boiling for ten hours with hydrochloric acid 1·16 sp. gr. Most of the acid was then removed on a water bath, and the remaining liquid taken up with water and distilled with magnesium oxide.

The ammonia liberated was equivalent to 13·8 c.c. N/10 acid, equal to 1·94 per cent. ammonia in the protein.

Tryptophane.

This was found to be present in the protein by the method of Hopkins and Cole[1].

Conclusions.

The results of the hydrolysis are given in the following table:

			Per cent. present
Glycine	page 366	·016 gm.	
Alanine	„ 366.....2·39	2·99 gm.	1·03
	„ 367.....0·6		
Valine	„ 372	37·0 gm.	12·71
Leucine and isoleucine	„ 365.....0·57		
	„ 367.....0·43		
	„ 368.....0·44	11·57 gm.	3·97
	„ 372.....9·0		
	„ 374.....1·13		
Proline (p. 367) 8·73 − 0·44 gm. Leucin =		8·29 gm.	2·85
Phenyl alanine ...	page 365	12·06 gm.	4·14
Aspartic acid ...	„ 372.....3·55	4·79 gm.	1·65
	„ 373.....1·24		
Glutaminic acid hydrochloride			
41·36	page 362		
0·46	„ 362		
0·26	„ 373		
42·08 equal to free acid		33·71 gm.	11·58

[1] *Jour. of Physiol.* 1901, Vol. 27, p. 418.

	Per cent.
Serine	present
Tyrosine	0·65
Arginine	6·06
Histidine	1·66
Lysine	1·19
Ammonia	1·94
Tryptophane	present
	49·43

The following points are also noteworthy:

1. It was found possible to separate practically the whole of the glutaminic acid as the hydrochloride by saturating the liquid resulting from hydrolysis with hydrochloric acid gas at 0° C., and keeping it for nine days alternately in an ice chest and in a freezing mixture. At the end of this time there was no perceptible increase in the amount of the crystalline deposit. In this way 11·35 per cent. of glutaminic acid was obtained. Subsequent examination of the esters after fractionation yielded only 0·23 per cent. It is therefore possible to make a practically complete separation of glutaminic acid by this method even if the protein contains as little as under 12 per cent.

2. The outstanding feature of the hydrolysis is the very high content of valine, 12·7 per cent. Most proteins yield on hydrolysis under 1 per cent. of valine, and with the exception of certain keratins the author can find no record of a protein yielding more than 2 per cent. The protein of linseed appears therefore to stand by itself in its high content of valine. It is possible that when the leucine and valine fractions of the amino-acid esters have been submitted to examination by Levene's method, considerable revision of the valine content may be necessary in many cases.

3. The percentage of tyrosine in the protein of linseed appears to be exceptionally low. The great variation in the percentage of tyrosine in the same protein according to different authorities seems however to throw considerable doubt on the accuracy of the method of separation commonly employed. Thus Osborne[1] finds only about one-third as much tyrosine in zein as Kutcher[2] obtained from the same protein.

The author finds that basic lead acetate precipitates from neutral or faintly alkaline solutions containing tyrosine, *e.g.*, solutions containing mixed amino-acids obtained by hydrolysis of casein, a basic lead salt of

[1] Osborne and Clapp, *Amer. Jour. Physiol.*, 1908, xx. 484.
[2] Kutcher, *Zeit. für Physiol. Chem.*, 1903, xxxviii. 3.

tyrosine, $2Pb(C_9H_{10}NO_3)_2 . 5Pb(OH)_2$, which is practically insoluble. This salt has been analysed and found to have the composition represented by the above formula—N = 2·44 per cent., Pb = 61·98 per cent. It is precipitated in a granular state and is readily filtered and washed. Using solutions of purified tyrosine, the author has been able to recover in the precipitate 87 per cent. of the nitrogen of the tyrosine. By decomposing the precipitate with sulphuretted hydrogen very pure tyrosine has been obtained. The writer is still investigating this subject with a view to working out a more accurate method of separating tyrosine.

"FATNESS" AS A CAUSE OF STERILITY.

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THE general experience of breeders, supported by statistical evidence in the case of sheep, has shown that a good thriving, but not over-fat, condition during the breeding season is that which is most conducive to a high fertility. It has been shown further that the practice of "flushing" or "springing" ewes (*i.e.* artificially stimulating them by means of an extra supply of special food) shortly before and during tupping time results in an increased number of births at the succeeding lambing time. On the other hand, an excessive quantity of nutriment producing a great deposit of fat is known to be prejudicial to the proper discharge of the reproductive functions. No better example could be given of the way in which overfeeding results in a condition of sterility than that of the barren shire mares which have been a noteworthy feature at many agricultural shows. It is a matter for regret that those animals, whose appearance justified them as prize-winners, should in many cases have proved valueless as brood mares owing to the methods of feeding to which they had been subjected in preparing them for show. The association of sterility with a too fattening diet is as common among cows, sheep and pigs as it is among mares. Thus Cornevin writes: "Chaque année, dans les concours, nous avons sous les yeux des spécimens des plus belles races ovines et porcines qui, véritable modèles de bonne confirmation, de puissance assimilatrice et d'aptitude à prendre la graisse, restent stèrile[1]." Moreover, Mr Edward Brown, the honorary secretary of the National Poultry Organisation

[1] Cornevin, *Traité de Zootechnie*. Paris, 1891.

Society, ascribes the decrease in egg production by poultry during the winter months in part at least to overfeeding. "In the late summer," he says, "it is natural for animals and birds to store up reserves of fat in anticipation of the winter. Until such reserves are exhausted functional activity is retarded. To feed heavily with a view to increased production is simply adding to the stores of fat and delaying ovarian action[1]."

Sterility, whether partial or complete, may be caused by an overfed condition in male animals as well as in females. Thus, according to certain veterinarians, an incapacity to procreate may be due to a deposition of fat in the muscles concerned in penile erection, which consequently cannot occur. This condition however is not necessarily permanent but may be remedied by starving. In such cases it would seem probable that the other generative organs are also affected. Dr Kisch of Marienbad states that in corpulent men the spermatozoa in the semen are fewer in number, and that in nine per cent. of the cases examined by him they were absent altogether.

Kisch states also that whereas the general proportion of sterile to fertile marriages is one in ten, in cases where the wife is very fat or both husband and wife are very fat it is increased to one in five[2].

It was the object of the investigation described herein to obtain information regarding the probable causes of sterility in fat animals, and more particularly in fat heifers. For this purpose the generative organs (ovaries, Fallopian tubes and uterus, and sometimes also the vagina) were obtained from a number of newly killed animals which were for the most part in the condition described by the butchers as "fat-ripe," and were often known to have been sterile for some time before killing. After being examined fresh the tissues were preserved in formaline (10 %), and were then prepared for histological study in the usual way.

The number of heifers examined was seven. Excepting for the last they all resembled one another by being in a distinctly fat condition. No. 7 was only moderately fat. In each of the other animals there was a considerable accumulation of fat around the internal genital organs, and more particularly the ovaries and Fallopian tubes. None of the animals were pregnant. The organs of the individual animals may now be described.

(1) This was an Aberdeen Angus heifer (named "Proud Mona"),

[1] Letter in *The Daily Express*, September 10th, 1910.

[2] Cooper, *The Sexual Disabilities of Man*, London, 1908.

aged two years and eleven months. She weighed 15 cwt. 8 lbs., and was highly commended at Smithfield Show, 1908. She was bred and exhibited by Mr J. J. Cridlan, of Maisemore Park, Gloucester. She failed to breed owing to her fat condition, her heat periods being few and irregular (at any rate latterly). A histological examination of the ovaries showed that they contained numerous Graafian follicles in all stages of development, some exhibiting a considerable degree of protrusion. The left ovary contained a corpus luteum which appeared to have been fairly recently formed. There were, however, also present a certain number of degenerate follicles, in which the epithelium had disappeared, partly or entirely, and in some of which the cavities were in process of being filled by ingrowing fusiform connective tissue cells derived from the ovarian stroma. The interstitial cells contained fat, and in one position there was a small patch of orange-coloured lipochrome. The uterus showed indications of former haemorrhage, presumably of a prooestrous nature, but otherwise did not appear to be abnormal.

(2) This was a fat heifer killed in Cambridge. The left ovary contained a corpus luteum and a few developing follicles of small size. The right ovary also contained follicles in various stages of development. Degenerate follicles were present in both ovaries. In the smaller ones the follicular epithelial cells and ova were still present, but had undergone varying degrees of atrophy. The ova were irregular in shape and in some cases were hardly recognisable. In the larger atrophic follicles the follicular epithelial cells and ova had disappeared but there was very little ingrowth of connective tissue from the walls. Bright red or orange-coloured lipochrome occurred in irregularly shaped spots or patches of considerable size in either ovary. This lipochrome was contained in large luteal-like interstitial cells. The patches were very striking on cutting the organs into pieces preparatory to histological investigation, but they could often be seen on the surface of the entire ovary. The uterus was normal, and showed no signs of previous prooestrous haemorrhage or pigment deposition such as are so often found in Ruminants during the breeding season.

(3) This animal was similar. The left ovary contained a normal corpus luteum but no visibly protruding follicles. The right ovary contained several moderately large follicles inside but none protruding from the surface. Fat and orange-coloured lipochrome were fairly abundant in the interstitial cells of the stroma in either ovary.

(4) This animal showed an old corpus luteum in the left ovary. Some of the luteal cells were vacuolated, showing that they were of

considerable age. The ingrowth of connective tissue was very far advanced, but large strands containing blood vessels were still apparently in process of growing inwards. In the rest of the ovary ova were very scarce. The other ovary contained no corpora lutea but a few follicles were present. Patches and spots of bright orange lipochrome were fairly numerous. The uterus was apparently normal.

(5) In this heifer there were no protruding follicles in either ovary, but lipochrome was abundant in the stroma. The left ovary contained a fully formed corpus luteum.

(6) In this animal there were also several brilliant lipochrome patches in each ovary. A follicle that seemed to have recently ruptured was found in the left ovary, and there was a normal corpus luteum in the right. The latter also contained a large degenerate follicle.

(7) I was able to obtain the breeding parts of this heifer through the kindness of Mr F. N. Webb of Babraham to whom I would express my indebtedness. She differed from those described above in being only moderately fat at the time of killing. She was born on July 19th, 1906, and had had two calves, the first on August 20th, 1908, and the second on May 29th, 1909. The latter was premature and should not have been born until August 1st. She was subsequently served on ten different occasions and by six different bulls but failed to hold each time. She also came in use at other times at intervals of three weeks. She was eventually killed on September 1st, when the generative organs were examined and preserved. The cervix uteri was found to be excessively constricted, a fact which by itself would be sufficient to account for the sterility. Moreover the uterine mucosa had undergone much fibrosis and the cavity of the organ was almost obliterated. On the other hand, the ovaries were nearly normal. The right one contained a very recently formed corpus luteum, a number of follicles in various stages of development, and no lipochrome in the interstitial cells. The left ovary also contained numerous follicles, but there were a few small patches of bright red or orange-red lipochrome in certain of the interstitial stroma tissue, but considerably less than in the ovaries of most of the animals described above. It seemed obvious that the sterility in this heifer was of a different order from that of the fat animals with abundant ovarian lipochrome.

It is well known among breeders of animals that females which are very fat do not come in use at all regularly and that in many instances the heat periods only occur occasionally or at very long intervals of time. Wallace remarks on this fact, pointing out that such animals

"settle better and feed faster as they become what the butchers designate as fat-ripe[1]." Mr K. J. J. Mackenzie tells me that within his own experience a somewhat underbred Hackney mare, which was purposely kept very fat for an old gentleman to ride, only came in use twice in the course of two years. Subsequently when much reduced her oestrous periods recurred with perfect regularity. So, similarly, two other mares under Mr Mackenzie's observation had to be half starved and got into a thin condition before conception was rendered possible, but when in this condition their oestrous periods became regular, and in due course they stood to the horse and subsequently foaled.

Experiments have shown that the recurrence of the oestrous cycle in animals is dependent upon the functional activity of the ovaries. After ovariotomy or spaying, provided that this operation is complete, heat can no longer be experienced. It has been demonstrated further that the ovarian influence in this respect is chemical rather than nervous, since in animals in which the ovaries have been transplanted to abnormal positions, and their ordinary nervous connections severed, they still retain their usual influence upon the other generative organs and upon the secondary characters of sex[2]. It would seem, therefore, that the recurrence of the oestrous cycle is dependent upon the elaboration of an internal ovarian secretion which, circulating in the blood, acts as a chemical excitant to the tissues of the other sexual organs and structures. According to Limon[3] the epithelioid interstitial cells of the ovary probably constitute the tissue concerned in manufacturing the secretion, and it is to be noted that it was in these cells that the lipochrome was deposited in the cases of sterility described above.

Excepting for the yellow pigment lutein which is formed in the epithelial cells of the corpora lutea and is sometimes classed as one of the lipochromes, these substances do not appear to have been described in the mammalian ovary. A pink lipochrome, however, is known to occur in the ovaries of the salmon, being transferred thither along with fat from the muscles at the approach of the breeding season when the fish migrate up the rivers from the sea. Lipochromes have also been found in the ovaries of certain Crustacea and other Invertebrates

[1] Wallace, *Farm Live Stock of Great Britain*, Edinburgh, 1907.

[2] Marshall and Jolly, "Contributions to the Physiology of Mammalian Reproduction," *Phil. Trans.* B. Vol. CXCVIII. 1905.

[3] Limon, "Observations sur l'État de la Glande Interstielle," &c., *Jour. de Phys. et Path. Gén.* Vol. XVI. 1904.

and often in the eggs, where their presence in such forms must be regarded as normal[1].

Speaking generally, lipochromes occur in association with fat but this is not invariable. Like fat they are soluble in alcohol, as could readily be observed when dehydrating the heifer's ovaries preparatory to making sections. Moreover, the sections from which the lipochrome had not been removed gave the characteristic indications of fat when stained for histological examination.

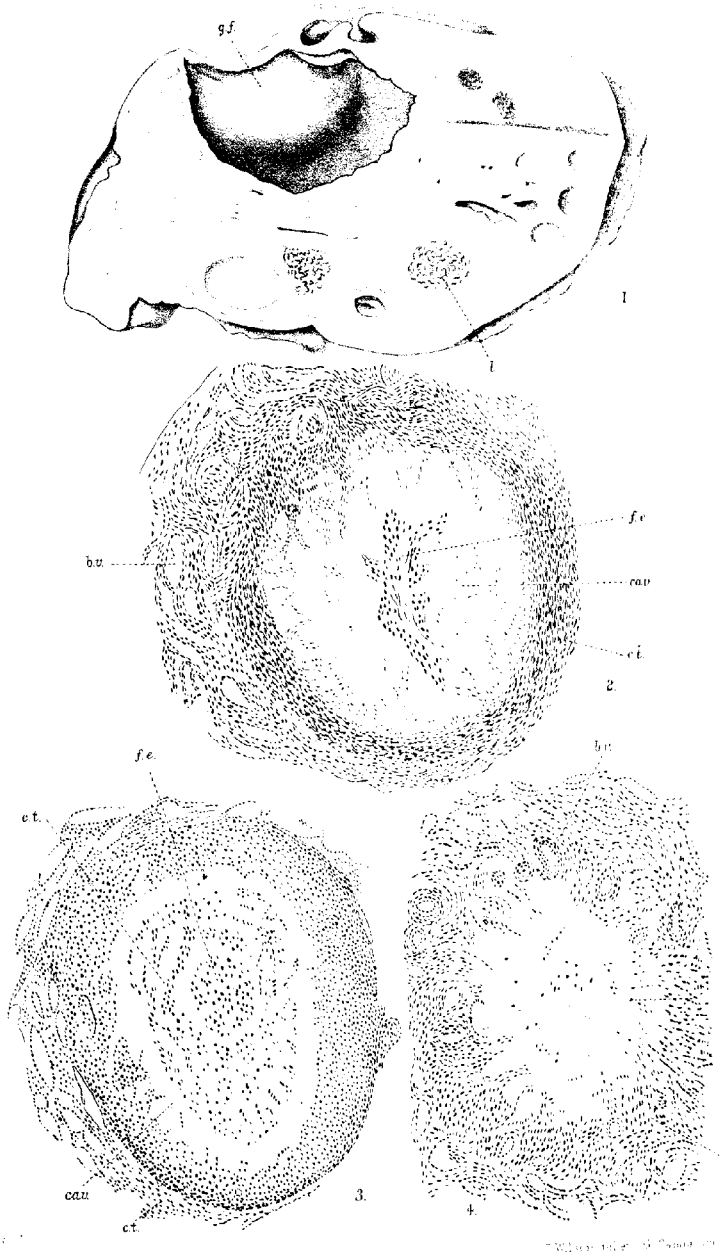
According to the observations of Miss Lane-Claypon[2] the interstitial cells and follicular epithelial cells of the ovary are potentially similar, both being derived from the original germinal epithelium, and van der Stricht[3] has shown that both of these cellular elements may take part in the formation of the corpus luteum. This structure, as is well known, is formed after ovulation and is apparently a factor in maintaining the raised nutrition of the uterus during pregnancy. If, however, pregnancy does not supervene the corpus luteum, after undergoing hypertrophy for a few days, becomes reduced in size and degenerates before the next heat period. It is clear, therefore, that no newly developed corpus luteum is present in the ovary during the prooestrum, and thus it is possible that the changed ovarian metabolism consequent upon the presence of the corpus luteum may have an inhibitory influence upon the occurrence of heat. If this is so, it would seem probable that the deposition of lipochrome in the epithelioid interstitial cells (associated as it is with the absence or great irregularity of heat periods) may have a similar influence upon the metabolism of the ovary to that normally exerted by the lutein deposited in the corpus luteum.

Next to the presence of lipochrome the most noteworthy characteristic of the fat heifers' ovaries was the number of degenerate follicles. These were in various conditions of atrophy as described above. The figures represent some of the later stages of degeneration whence the original follicular cavities with their serous contents are in process of being filled in by connective tissue growing from the wall in the manner shown. The ova had completely disappeared in these follicles, but epithelial cells were still sometimes present. It has been shown

[1] Miescher, *Histochemische und Physiologische Arbeiten*, Leipzig, 1897. Maly, "Über die Dottenpigmente," *Ber. des Akad. des Wissensch. in Wien*, Vol. LXXXIII. 1881. Krukenberg, *Vergleichende phys. Studien*, Heidelberg, 1882. Heim, "Sur les Pigments des Œufs des Crustacés," *C. R. de la Soc. de Biol.* Vol. XLIV. 1892.

[2] Lane-Claypon, "On the Origin and Life History of the Interstitial Cells of the Ovary of the Rabbit," *Proc. Roy. Soc.* B. Vol. LXXVII. 1905.

[3] Van der Stricht, "La Rupture du Follicule Ovarique et l'Histogénèse du Corps Jaune," *C. R. de l'Assoc. des Anatomistes*, 3rd Session, Lyon, 1901.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in rabbits and other animals that follicular degeneration may result from failure to ovulate and that if undergone on an extensive scale and for a considerable period it induces eventually a condition of permanent sterility[1]. It would seem probable, therefore, that follicular degeneration arising from badly regulated nutrition (whether in cows or other animals) may also lead to barrenness, at any rate of a temporary kind.

Lastly it is always possible that in cases of extreme "fatness" sterility may be caused simply by mechanical factors such as the blocking or anatomical derangement of the Fallopian tubes, and the consequent incapacity of the ova to gain access to the tubes, owing to an excessive deposition of fat in that region. Such an explanation, however, could scarcely be applicable to more than one of the cases above considered, (namely the first), while it was by no means certain even in this instance that a discharged ovum could not have gained access to the oviduct.

The general conclusions reached are that the derangement of the oestrous cycle in fat animals is caused by a disturbance of the ovarian metabolism as manifested especially by a considerable deposition of pigmented fat or lipochrome in the interstitial tissue, and that this process is accompanied by an unusually extensive degeneration of follicles which may lead to a prolonged state of sterility. However, since the degeneration does not usually occur to any especially great extent in the smaller or less mature follicles, it may be inferred that the sterility so induced is commonly of a transient nature, and can be remedied sooner or later by a reduction in the quantity of food supplied or by an increase in the amount of exercise. And this conclusion appears to accord with the results of actual practice.

DESCRIPTION OF PLATE.

Fig. 1. Section through ovary of fat heifer somewhat magnified. *g.f.*, Graafian follicle. *l.*, lipochrome (the original colour being reproduced).

Fig. 2. Section through degenerate follicle showing epithelial cells (*f.e.*) in a state of partial disintegration, and connective tissue (*c.t.*), some strands of which are in process of growing inwards to fill up the cavity (*cav.*). *b.v.*, blood vessel.

Fig. 3. Section through degenerate follicle showing the remains of the follicular epithelium (*f.e.*) surrounded by ingrown connective tissue (*c.t.*). *cav.*, cavity. The central connective tissue is derived from the wall of the follicle, but no indication of this is shown in the figure.

Fig. 4. Section through degenerate follicle. *b.v.*, blood vessel. *cav.*, cavity. *c.t.* connective tissue from which ingrowing strands are derived. The follicular epithelium has very nearly disappeared.

[1] Heape, "Ovulation and Degeneration of Ova in the Rabbit," *Proc. Roy. Soc.* B. Vol. LXXVI. 1905.

A BACTERIAL DISEASE OF SWEDES.

By J. H. PRIESTLEY, B.Sc., F.L.S. (*Lecturer in Botany, University of Bristol*) and A. E. LECHMERE, B.Sc. (*London*), M.Sc. (*Bristol*).

Introduction.

In the early part of April, 1910, specimens of swedes were sent from the neighbourhood of Taunton to the Botanical Department at the University of Bristol for examination.

Some of the specimens sent were attacked by *Plasmodiophora brassicae*, the cause of the common finger and toe disease, and showed the appearance characteristic of this attack, but others, while often in an advanced stage of decay, showed no indication of the presence of this disease and the roots retained their normal shape.

In the latter case the first indication of attack was described as being the appearance of a small crack on the side of the root, almost as if made by a hoe; this crack gradually widened, the interior being filled with slimy rotting tissue; the continued progress of the disease in many cases caused the death of the plant.

In the previous autumn the field had been infested with surface caterpillars, and eelworm had lately been noticed in the swedes, while some showed traces of attack by wireworms. The prevalence of finger and toe in the same field seemed to point to an absence of lime in the soil.

The disease appeared chiefly in one large field in which two varieties of swede had been sown: the soil is a loam, in most parts well drained, dry and shallow, but with local poorly drained damp areas. Both varieties were affected by the disease and apparently to the same extent, and the disease was spread evenly over both dry and damp areas of the field.

The tissue exposed in the cavities in the roots, the formation of which has been described, was discoloured and very soft, having the

appearance of a light-brown slime. On microscopical examination the slime was found to consist of numerous motile bacteria and protozoa. Sections of the diseased root were cut by hand and some material was fixed and embedded in wax for the microtome.

The hand sections showed the gradual decay of the cells and disintegration of the cell wall; some sections were obtained where the bacteria were swarming in the intercellular spaces and in a cavity in the cell wall formed by the decay of the middle lamella. This gave very much the appearance of the disease described by Potter and identified by him as *Pseudomonas destructans*. The action on the cell wall and the motility of the bacteria, facts upon which he lays stress, were both very marked.

To prove that the destruction of the tissue was due to bacterial action and not to animal attack alone, some of the slime from an infected root was added to pieces of fresh swede and turnip, kept moist in Petri dishes. In the course of a few days the infected pieces of root developed a decaying area of slimy tissue which showed the presence of abundant bacteria. Attempts were then made to isolate the organisms, and with a view to doing this, a medium, consisting of turnip extract and gelatin was prepared as follows. One large turnip was pared with a knife, cut in pieces, and put through a mincing machine, the juice was collected and the minced turnip pressed in a screw press. The extract thus obtained was made up to 500 c.c. with distilled water, and allowed to stand in the incubator at 25° C. for some hours, it was then filtered. Half was neutralized with sodium carbonate, the other half was left naturally acid, each quantity was then made up to 10% gelatin, and poured off into tubes, which were sterilized in the usual way. A second turnip was steamed till soft, then cut into blocks, which were placed in tubes and sterilized at the same time as the gelatin medium. The following method was then used for the isolation of the organisms:

A tube of naturally acid medium, previously melted, was inoculated with a loopful of liquid slime taken from a diseased swede. After shaking well this tube was used to pour a series of eight plates in the usual manner. After a few days the colonies developed, the first three plates very soon liquefied as the number of colonies was very great. On the last four plates the colonies were sufficiently separated to enable isolations to be made.

Four colonies were isolated from this series of plates. Three of these colonies produced liquefaction of the gelatin, and on examination

were found to consist of motile bacteria. Each of these colonies showed a characteristic colour, and was distinguished by a separate reference letter—*B* (*a*), *B* (*b*), *B* (*c*).

B (*a*) produced a magenta secretion which coloured the medium on which it was cultivated. The gelatin became bright magenta and formed a brilliantly coloured liquid after nine days growth. On potato blocks the colour was most brilliant, and on turnip blocks the secretion coloured the whole block and stained the cotton wool plug at the bottom of the tube.

B (*b*) formed a whitish colony and grew rapidly on gelatin streaks, on neutral tubes liquefaction took place in 4—5 days, but in naturally acid tubes liquefaction was not produced till after 7 or 8 days at room temperature. It grew well saprophytically on sterilized turnip blocks.

B (*c*) produced liquefaction in gelatin streaks but more slowly than the other forms, the colonies had a yellow colour.

The fourth organism, *C*, appeared as a dense white colony growing on the surface of the gelatin, and producing no liquefaction; it was found to be a yeast, small, spherical, and with a single highly refringent granule.

From a second series of plates two more organisms were obtained and isolated as *A* and *D*. *A* formed an opalescent semi-transparent colony, growing well on gelatin streaks and producing liquefaction only after some time. On the sterilized turnip blocks it formed a slimy film, which on examination was found to consist of non-motile bacilli, possibly a zoogloea condition with a gelatinous matrix.

The last organism, *D*, formed a salmon pink colony and grew well on gelatin streaks and on turnip blocks, forming a smooth surface of salmon pink colour. On microscopic examination it proved to be a yeast with somewhat elongated cells.

In addition to these organisms, the presence of numerous protozoa was noted in all cases where raw turnip had been inoculated with slime from a diseased turnip: these cannot be obtained in pure culture, but when a streak was made directly from liquid slime the culture invariably showed a mixture of bacteria, yeast and protozoa.

Two forms of protozoa were found, and stained preparations obtained to show the structure. The first form consisted of a rather large colourless body, slightly pointed at the anterior end to which two long equal terminal flagella were attached.

The second form had an elongated colourless body, very contractile,

and apparently only one single flagellum. As nearly all the protozoa are saprophytes, the presence of these forms may be attributed to secondary infection, the decaying cell substance would provide an excellent medium for their development. (These protozoa certainly did not represent the swarmer stage of such a parasitic Myxomycete as Plasmodiophora.)

The action of all the organisms isolated was tried on sterilized turnip blocks, in all cases growth occurred, but in no case was any discolouration or sign of decay produced except in the case of *B* (*b*), which after some time produced a decided softening of the tissue, probably due to action on the cell walls. The absence of discolouration may be accounted for by the destruction of all enzymes in the turnip on sterilization, at the same time a watery extract would be formed all over the surface of the block, which would provide a suitable medium for the growth of the organisms as saprophytes over the surface.

More direct evidence as to the parasitic nature of *B* (*b*) was obtained in experiments in which raw turnips were employed. A well-cleaned turnip was cut into thin slices about 1 cm. thick with a knife, which had been cleaned in methylated spirit and then in sterilized water. The pieces of turnip were then quickly transferred to sterilized Petri dishes and moistened with sterilized distilled water; one plate in each set so prepared was kept as a control, and the other plates inoculated with pure cultures of the various organisms. The action of these organisms upon the turnip blocks is given, in tabular form, below:

A after 3 days formed gelatinous mass on surface, tissue remained hard.
B (*a*) after 3 days formed coloured mass, tissue remained hard.
(*b*) ,, ,, ,, discoloured patch, tissue became soft.
(*c*) ,, ,, ,, slight growth, tissue remained hard.
C after 3 days formed a slight growth, tissue remained hard.
D ,, ,, ,, ,, ,, ,, ,, ,,

A mixture of all the organisms showed discolouration and softening of the tissues in parts.

The following method of staining was used for comparison of the various organisms as regards microscopical appearance.

A film was spread with a platinum loop and dried on the slide at air temperature; the slide was then covered with weak chrom-acetic acid for 10 minutes, drained, washed in distilled water 3 minutes, drained, then the stain applied to the slide. Aqueous anilin gentian violet was used, and allowed to act for 3 minutes, drained off, washed

in water, drained and dried with filter paper, warmed over a flame, balsam and a clean cover-glass added.

By this method good detail was obtained. The protozoa showed the flagella distinctly and some internal differentiation. The yeast *D* showed granular structure and no vacuoles, while *C* showed a deeply staining granule and a vacuole.

The bacteria *B* (*a*), *B* (*c*), stained as small simple rods, but *B* (*b*) showed polar staining.

From the results obtained from inoculation experiments on turnip blocks it would seem that the particular organism causing the disease is that described as *B* (*b*), while several facts seem to indicate that the organism isolated as *A* is only a form of *B* (*b*), as when *B* (*b*) is grown on agar streaks, it much resembled the opalescent colonies of the *A* form on gelatin. Also, when the *A* form has been grown on a gelatin streak for some time its appearance is exactly the same as that of *B* (*b*) when only a few days old. It may be a zoogloea condition of *B* (*b*), as cultures of this show this condition in stained preparations. This might also account for the gelatinous slime so often present in inoculated turnip blocks.

A further series of experiments were carried out on seedlings of turnip and swede grown in pots, about five seedlings in each pot. These were inoculated with pure cultures of *B* (*b*) in the motile form. The cultures were supplied in the form of suspensions in distilled water, to which a quantity of a pure culture had been added by means of a platinum loop. The water tubes were incubated for some hours at 25° C. before using for infection.

Inoculations were made on injured and uninjured seedlings and control sets kept in each case. The injuries were made in three ways: (1) wounding the stem at the ground level with a blunt scalpel, (2) piercing the stem with a needle dipped in the culture liquid, (3) piercing the leaf of the seedling with an infected needle.

The results of these experiments lead to no positive conclusions, although many of the inoculated plants which had been wounded with a blunt scalpel died off, several which had not been inoculated suffered the same fate.

As the seedlings are very delicate and it is impossible to wound each to the same extent this cannot be taken as conclusive.

The reason for the failure of the cultures to obtain sufficient hold on the seedlings is probably that given by Dr E. F. Smith and other workers who assert that only injured roots can be attacked. The

bacteria must then live for some time in the injured tissue as saprophytes, and so attain sufficient strength by rapid growth and increase of numbers to enable them to penetrate the tissue as parasites. In the case of the seedlings the wounded area must necessarily be small, and would probably heal up before the bacteria could obtain sufficient hold.

Hanging drop cultures in Ward's tubes were made with sections of raw turnip and of swede, in water, which were then infected with cultures of *B* (*b*), the bacteria were seen actively swarming round the tissue and after some time were found inside the cells, and in the intercellular spaces, although the actual penetration of a wall has not been observed.

Attempts were made to stain the flagella by the Löffler flagella stain, and some by van Ermengen's method. No sign of a single terminal flagellum could be found, but the preparations showed clearly the existence of peritrichous flagella. With the Löffler stain good results were obtained, while the van Ermengen method did not prove satisfactory, our experience being therefore the reverse of that of Potter.

In a recent paper by Johnson a comparative account of the bacteria producing the various forms of rot in turnips and other cruciferous plants is given. They are as follows:

(1) *Brown Rot.* Caused by *Pseudomonas campestris* (Smith). The attack proceeds slowly causing blackening of the tissue, which remains hard and dry. Infection always through a wound or crack, possibly produced through over manuring the crop, the organism follows the course of the vascular system, and is a motile bacillus with a single terminal flagellum; it forms yellowish colonies and liquefies gelatin.

(2) *Soft White Rot.* Caused by *Pseudomonas destructans* (Potter), its attack proceeds more quickly than that of "Brown Rot," causing softening of the tissue which becomes yellowish and strongly smelling. The organism is a motile bacillus with a single terminal flagellum, aerobic, liquefies gelatin (peptonising ferment), softens cell wall (cytase), hydrolyses starch (diastase), gelatin colonies whitish-grey, stab culture produces a funnel-shaped depression filled with liquid gelatin with a cloudy deposit, white glazy growth on agar, parasitic on turnip, potato and carrot, cloudy appearance in turnip broth, produces oxalic acid in turnip juice and in sugar media, residual products acid, gives off CO_2 during fermentation. Stains with anilin dyes, stained by Gram's method shows polar staining.

(3) *Soft Black Rot of Cabbages.* Caused by *Bacillus oleraceae* (Harrison), this organism agrees very closely on all points with *Pseudomonas destructans* (Potter) and only differs in having (1) a white opalescent growth on agar, (2) 7—13 peritrichous flagella instead of a single terminal flagellum, and (3) a slight difference in size, which however seems to be variable.

The organism isolated as *B(b)* has been grown under as many conditions as has been possible in the limited time available for its investigation, and has been found to agree with the two closely allied organisms *Pseudomonas destructans* and *Bacillus oleraceae* on the following points:

Actively motile bacillus, aerobic, liquefies gelatin streaks, forms whitish colonies on plates, produces a funnel-shaped depression in stab cultures with a cloudy appearance in the liquid gelatin, forms a white opalescent growth on agar (and sometimes on gelatin). Grows well on turnip and carrot, and produces softening of tissue showing cytase action on cell wall, produces acid reaction in neutralized media, gives off gas during fermentation, hydrolyses starch, showing diastase action, shows marked polar staining, stains well with anilin dyes, and is stained by Gram's method.

It resembles *Bacillus oleraceae* (Harrison) on two of the points in which this organism is distinct from *Pseudomonas destructans* (Potter), viz.:

(1) The peritrichous flagella.

(2) The opalescent colonies on agar (and sometimes on gelatin).

At the same time it hardly seems likely that these distinctions are of permanent value, the size of the organism changes under cultivation, and it seems quite probable that this may be accompanied by changes in the general appearance (opalescence) of the colonies and also, possibly, by changes in the number and attachment of its flagella[1].

The cultures described above show that the organism is capable of existence as a saprophyte, and this means that it may continue to exist in the soil, on rotting tissue, long after the crop itself has been removed. This makes the question of the extermination of the disease a difficult one; one obvious precaution is to lengthen the period as far

[1] Our conclusions seem in agreement with those of Harding and Morse, Bulletin No. 147, Vermont Agricultural Experimental Station, but unfortunately this paper did not reach us until our own work was completed.

as possible between the successive cruciferous crops, when the disease has once appeared. It seems capable of growing both in alkaline and acid media, so that it does not seem clear that any particular dressing applied to the soil, should affect its growth. In this particular case of infection the disease was prevalent at the same time and under the same conditions as finger and toe, conditions which indicate the presence of an insufficient quantity of lime in the soil. The practical question, as to the best treatment of the soil, seems only capable of attack upon a large scale, by actual experiment in the field.

Summary.

A disease of swedes is described and attributed to the action of an organism, probably *Bacillus oleraceae* (Harrison), but closely allied to *Pseudomonas destructans* (Potter). It is suggested after consideration of its appearance on various culture media, that these two organisms may be different growth forms of the same parasitic species.

REFERENCES.

HARRISON, F. C. "A Bacterial Disease of the Cauliflower and Allied Plants." *Cent. für Bakt.* Abt. II. Band XIII. 1904.

JOHNSON, T. "Bacterial Rot in Turnips and other Brassicas of Ireland." *Econom. Proc. of Roy. Dublin Soc.* Vol. II. No. 1, Feb. 1910.

MIDDLETON, T. H. "Black Dry Rot in Swedes." *Journ. Board Agr.* Vol. IX. 1902.

POTTER, M. C. "On Brown Rot of Swedish Turnip." *Journ. Board Agr.* Vol. X. Dec. 1903.

—— "On a Bacterial Disease 'White Rot' of the Turnip." *Proc. Univ. Durham Phil. Soc.* Nov. 1899.

—— "On a Bacterial Disease of the Turnip." *Proc. Roy. Soc.* Vol. LXVII. 1901.

—— "On the Parasitism of *Pseudomonas destructans*." *Proc. Roy. Soc.* Vol. LXX. 1902.

SMITH, E. F. "*Pseudomonas campestris* the cause of 'Brown Rot' in Cruciferous Plants." *Centralblatt für Bakteriologie*, Abt. II. Band III. 1897.

—— "The effect of Black Rot on Turnips." *U.S. Dept. of Agr. Bureau of Plant Indust.* Bull. 29, 1903.

NOTE ON THE COMPOSITION OF SOOT.

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The value of soot as a manure depends upon the ammonium salts which it contains, as well as upon its beneficial effect on the texture and colour of the soil, and its power of diminishing the ravages of slugs and small snails upon a young crop. In various samples which from time to time have been submitted to analysis the percentage of nitrogen present has been found to vary within very wide limits, from 0·5 to 7 per cent., and it is usually stated to be present in the form of ammonium sulphate; actually it occurs for the most part as ammonium chloride.

The following investigation was carried out in order to throw some light upon the relation of the general characteristics of a sample of soot to its nitrogen content. The nitrogen was estimated by Kjeldahl's method, the values being very slightly higher than those obtained by directly distilling the soot with caustic soda. The discrepancy is probably not due to the existence of non-ammoniacal nitrogen in the soot, but to the raw soot not getting readily wetted by the alkaline solution. Eleven samples were collected and examined with the following results.

Amongst samples taken from dwelling houses, no relation was found between the nitrogen content and either the excellence of the draught in the chimney or the percentage of ash in the soot.

Flue dust of a characteristic reddish colour from a tall boiler chimney was found to contain only 0·5 per cent. of nitrogen and 75 per cent. of ash. A reddish colour in itself however is no indication of a low nitrogen content, since soot from a kitchen chimney with 5·4 per cent. of nitrogen had a distinctly reddish tinge.

Flue dust from the Cambridge Rubbish Destructor, similar to that from the London Destructors used extensively upon the hop-fields of Kent, contained no nitrogen and was composed almost entirely of

inorganic matter, mostly oxides of aluminium and iron with calcium carbonate.

A sample of soot consisting of flakes of tarry matter from a wood fire was found to contain 6·4 per cent. of nitrogen, a distinctly high value.

It seems most probable that the nitrogen content of soot from dwelling houses depends chiefly on the kind of coal burnt. Since soot as sold is made up of a little from one house and a little from another, it would be of small utility to investigate this point.

The following table shows a relation between the nitrogen content and the weight per bushel of the soots examined. It further indicates that there is some loss when the soot is allowed to stand in a loose heap, as in the case of the three mixed samples. A small amount of ammonia was found to be given off when air was drawn through a tube containing soot.

Type of soot	Percentage of nitrogen	Lbs. of soot per bushel loosely packed	Lbs. of nitrogen per bushel of soot
From Kitchen chimney	11·0	9	1·0
From Sitting Room chimney ...	5·5	18½	1·0
From Kitchen chimney	5·4	23	1·2
From coal and wood fire	4·5	26	1·2
From dwelling houses (*mixed*)...	3·6	29	1·0
From low cottage chimney	3·14	33	1·0
From dwelling house (*mixed*)...	2·9	28	·8
From dwelling houses (*mixed*)...	2·7	27	·7
From 40 ft. boiler shaft	·5	47	·23

It has been generally accepted by farmers that a light soot is the best; this is well borne out by the above table. This points to the conclusion that soot should be bought by volume and not by weight, for then the buyer is more likely to get an approximately constant quantity of nitrogen per bushel. Further if soot has been adulterated with ashes or grit this will have less effect upon the volume than upon the weight. Nitrogen in the form of readily available plant food is worth about sixpence a pound, hence a bushel of fresh light soot is worth about sixpence from its fertilising value alone, regardless of its beneficial action upon the soil and its special power of deterring the visits of insect pests.

The analyses were made in the School of Agriculture at the suggestion of Professor T. B. Wood, to whom and to Mr F. W. Foreman, I desire to express my thanks for advice.

THE FUNGICIDAL PROPERTIES OF LIVER OF SULPHUR.

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LIVER of sulphur, according to Thorpe[1], is an alchemistic term coming down to us from a time when there was no clear distinction between the alkalies, potash and soda. As a rule, the preparation was made from crude carbonate of potash, mainly for the reason that this substance was more easily procurable than soda. It was obtained "by fusing in a Hessian crucible a quantity of potassium carbonate with half its weight of flowers of sulphur, and the fused mass poured out on a greased flagstone and allowed to solidify[2]." From the peculiar liver-brown colour the product derived its name.

The pharmaceutical preparation known as "Liver of Sulphur" is supposed to be made from potassium carbonate, probably as a survival of the old state of things. In manufacturing this substance for horticultural purposes, some manufacturers now substitute sodium carbonate for the more expensive potassium compound, and this fact may account for the difference in price.

Liver of sulphur is of general use as a fungicide, and its employment has been much extended in recent years owing to the many attempts which have been made to check the ravages of American Gooseberry Mildew (*Sphaerotheca mors-uvae*). For the latter purpose it is used in large quantities, and its analysis is therefore a matter of considerable importance. Before an intelligent analysis can be made, it is necessary to know to which of the several constituents its fungicidal power is due. No evidence, however, on this point can be found.

Samples of the commercial product were collected from various makers, and sent to the writer by the Board of Agriculture and

[1] Private communication to the Board of Agriculture and Fisheries.

[2] Thorpe's *Dictionary of Applied Chemistry*, p. 471.

Fisheries, who first proposed the enquiry. Information was desired by them as to the composition of the material sold, and the reason or reasons for its fungicidal properties, with a view to discovering a suitable method for estimating the fungicidal value of any particular sample.

Description of Samples.

Attention will be confined to half a dozen samples, which for purposes of reference will be called *A*, *B*, *C*, *D*, *E*, and *F*.

A. Yellowish green colour, very deliquescent, almost completely soluble in water, alkaline to litmus.

B. White, very much less deliquescent than *A*, and not so alkaline to litmus, an insoluble residue left on treatment with water.

C, *D*, *E* and *F*. Similar in appearance to either *A* or *B*, with varying deliquescence, alkalinity, and amount of insoluble residue when treated with water.

Further examination of Samples.

With the exception of *B*, the samples all gave a distinct smell of sulphuretted hydrogen when moistened with water. On extracting each sample separately with water, filtering, and adding hydrochloric acid, the amount of sulphuretted hydrogen evolved and sulphur precipitated varied in each case. Sample *B* gave only a small quantity of sulphuretted hydrogen, with practically no effervescence. Sample *A* gave most, the effervescence being quite violent. Sulphur dioxide was also evolved in small quantity when the acid was added, particularly from Sample *A*, indicating the presence of thiosulphate or sulphite or both. Presence of thiosulphate was definitely established by boiling the water extract with zinc chloride to remove sulphides and polysulphides, and filtering. The filtrate instantly discharged the colour of a few drops of a solution of iodine in potassium iodide, and on adding hydrochloric acid to another portion of the filtrate, sulphur dioxide was evolved, and a small quantity of free sulphur precipitated. Evidence was also obtained of the presence of di- tri- and pentathionates. None of the samples contained lime.

The Polysulphides.

It was observed that the amount of sulphur precipitated on adding hydrochloric acid to a clear water extract of the fungicide was much

less than that demanded by the following equation, even in the case of those samples which left a residue of sulphur on treatment with water:

$$K_2S_5 + 2HCl = 2KCl + H_2S + 4S.$$

Liver of sulphur therefore must contain a mixture of the various polysulphides. It has been found impossible to obtain pure potassium pentasulphide, even when excess of sulphur is used; a mixture of polysulphides always results.

Free Alkali and Insoluble Matter.

Free Alkali. A very rough idea of the amount of free alkali was obtained by treating portions of 1 gm. with water till as much as possible dissolved, filtering and titrating the filtrate with deci-normal acid, with vigorous stirring until opalescence first appeared, indicating that the free alkali was neutralised and the acid had commenced attacking the polysulphides, liberating sulphur therefrom. The results are given in Table I.

TABLE I.

Free Alkali in portions of one gramme.

Sample	c.c. $\frac{N}{10}$ acid required before opalescence appeared
C	21·3
A	11·5
D	0·1
B	None. Rapidly turned milky with no addition of acid
E	" " " " " "
F	" " " " " "

Insoluble Matter. The insoluble residue was washed, dried, and weighed, and the percentages are given in Table II.

TABLE II.

Sample	Insoluble Matter per cent.	Appearance of residue
A	0·2	black
B	4·4	greyish-white
C	1·3	black
D	8·2	greyish-white
E	5·0	" "
F	9·9	dark grey

The light-coloured residues consisted of sulphur. The figures clearly reflect the lack of a standard method of manufacture. The amount of sulphur used relative to the alkali has varied considerably, or it may be that the insoluble residual sulphur has resulted from incomplete fusion. Evidently great variation exists between the various preparations on the market.

Estimation of Potash.

Water extracts of the samples were boiled with nitric acid, and bromine water added until all the sulphides and sulphur were oxidised, and the liquid was then made up to a known volume. An aliquot portion was neutralised with soda, a few drops of acetic acid added, and the potash precipitated by sodium cobaltinitrite solution. The percentages of potash are given in Table III.

TABLE III.

Sample	Per cent. K_2O
A	51·3
B	7·4
C	33·4
D	4·1
E	49·7
F	35·1

It will be observed that soda has been used in the manufacture of Samples *B* and *D*.

Constituents of Liver of Sulphur.

The following substances were found in the various commercial samples examined: free sulphur, free alkali, sulph-hydrates, sulphides, polysulphides, sulphites, thio-sulphates, and thionates of either potassium or sodium. When moistened, sulphuretted hydrogen in small quantity was evolved continuously.

To ascertain to what the efficiency of liver of sulphur as a fungicide is due, it was decided to study the effect of weak solutions of the various components upon the germination of spores of *Botrytis cinerea*. As most of the samples emitted a distinct smell of sulphuretted hydrogen when moistened, and all gave it when treated with acid, the effect of a saturated solution of this gas was also tried. Free sulphur is said to be

very valuable for checking certain mildews, and is very frequently used for this purpose. Most of the samples examined contained appreciable quantities of free sulphur, and sulphur was deposited from the water extracts, either at once or after a short time. Deposition of sulphur from the solutions when plants are sprayed would be facilitated by carbonic acid or traces of other acid substances which may exist on the leaves. Therefore sufficient grounds exist for the inclusion of free sulphur as a possible factor in the efficiency of the fungicide, and the effect of a mixture of flowers of sulphur and water was tried side by side with the other solutions.

Liver of sulphur is used at the rate of ½ oz. to a gallon of water, which corresponds to 0·33 per cent. The following substances were dissolved separately in water, and the behaviour of Botrytis spores was watched in solutions of each, ranging from 0·08 to 1 per cent.: Na_2S, Na_2SO_3, $Na_2S_2O_3$, KHS, NaOH, KOH, $Ca(OH)_2$, a solution of the mixed polysulphides with no oxidation products present, Sample *A*, Sample *B*, a saturated solution of H_2S at ordinary temperature containing 0·25 per cent. H_2S, and a mixture of flowers of sulphur with water.

The "solution of the mixed polysulphides" was obtained in the following manner: 50 per cent. potash solution was mixed with excess of flowers of sulphur and kept in a warm place for several days out of contact with air, the mixture being repeatedly shaken until it was judged that no more of the sulphur would be dissolved. Some of the red-brown liquid was then decanted off, and its contents of potassium and total sulphur estimated. It was found to contain 30·6 per cent. K and 44·7 per cent. S. On adding hydrochloric acid to some of this liquid, a small quantity of an oily substance appeared at the bottom. This was H_2S_2, which proves the presence of K_2S_2, at least in the original liquid. A little more than twice as much sulphur as potassium would have to be present to correspond to the formula K_2S_5. It is therefore practically safe to assume that the liquid consists of a mixture of the various polysulphides, with potassium sulph-hydrate and potassium sulphide also present. It was considered as such a solution throughout the experiments, and its effect upon Botrytis observed. As it was impossible to separate the individual polysulphides, two kinds of weak solutions were made from the original liquid, one based on the content of potassium, and the other on the content of sulphur. These will be termed "Polysulphides K" and "Polysulphides S" respectively.

Method of Procedure.

The spores of Botrytis were seeded into about 2 c.c. of the solutions contained in watch-glasses, and these were placed in tightly covered vessels with water at the bottom to keep the enclosed air saturated, thus preventing evaporation of the solutions. Care was taken to wet the spores with the solutions. The trials were all incubated at 22° C., and examined from day to day. Two control watch-glasses containing water only were included in each series. Hanging-drop and other methods were tried, but were found unsuitable, proper access to air being apparently essential to germination.

SERIES I. 0·33, 0·66, *and* 1·0 *per cent. Solutions.*

Particulars of Solutions			Behaviour of spores of *Botrytis cinerea*					
			20 hours		42 hours		120 hours	
No.	Composition	Strength per cent.	Number germinated	Length of hyphae in spore diam.	Number germinated	Length of hyphae in spore diam.	Number germinated	Length of hyphae in spore diam.
1	Distilled water	—	Many	10	all	long	all	very long
2	,,	—	,,	10	,,	,,	,,	,, ,,
3	H_2S	Satd.	,,	5	,,	,,	,,	,, ,,
4	Mixture of very fine flowers of sulphur with water	0·33	,,	10	,,	,,	,,	,, ,,
5	do.	0·66	,,	10	,,	,,	,,	,, long
6	KOH	0·33	very few	1	many	5	many	
7	,,	0·66	none		none		none	
8	NaOH	0·33	,,		,,		,,	
9	Na_2S	0·33	,,		,,		,,	
10	KHS	0·33	few?		no change		no change	
11	,,	0·66	none		none		none	
12	$Na_2S_2O_3$	0·33	many	5	many	8		
13	,,	0·66	,,	7	,,	20		
14	,,	1·0	none		very few		few	
15	Na_2SO_3	0·66			few			
16	,,	1·0	none		none		none	
17	Sample *A*	0·33	,,		two	2	many	
18	,,	0·66	,,		none			
19	Sample *B*	0·33	few	5	many	8	none	
20	,,	0·66	none		none			

Comments on Series I.

0·33 per cent. NaOH, Na_2S, and KHS have prevented germination. 0·33 per cent. solutions of KOH, and of Samples *A* and *B* were

apparently not quite strong enough. Saturated H_2S solution and mixture of flowers of sulphur and water had no effect whatever. The hyphae were seen under the microscope to be pushing right into contact with the particles of sulphur.

SERIES II. *Weaker Solutions.*

Particulars of Solutions			Behaviour of spores of *Botrytis cinerea*			
			19 hours		42 hours	
No.	Composition	Strength per cent.	Number germinated	Length of hyphae in spore diam.	Number germinated	Length of hyphae in spore diam.
1	Distilled water	—	Many	8	all	increased
2	,,	—	,,	8	,,	,,
3	H_2S	Satd.	,,	8	,,	,,
4	$Ca(OH)_2$	0·06	,,	8	,,	,,
5	,,	0·08	,,	8	about half	very long
6	,,	0·16	,,	8	,,	,,
7	Na_2S	0·08	,,	8	all	,,
8	,,	0·16	,,	2	nearly all	,,
9	KHS	0·08	,,	8	all	10
10	,,	0·16	few	2	nearly all	7
11	KOH	0·08	nearly all	3	all	very long
12	,,	0·16	some	2	many	4
13	NaOH	0·08	few	3	few	10
14	,,	0·16	none	–	none	—
15	Sample *B*	0·08	few	4	few	10
16	,,	0·16	very few	2	few	4
17	Sample *A*	0·08	many	7	many	8–10
18	,,	0·16	very few	1	few	3
19	Polysulphides K	0·08	few	4	few	10
20	,,	0·16	none	–	none	—
21	Polysulphides S	0·08	few	4	few	4
22	,,	0·16	one?	1	very few	1
			67 hours		88 hours	
22	Polysulphs S	0·16	very few	—	few	5
20	Polysulphs K	0·16	none	—	none	—
18	Sample *A*	0·16	few	—	few	—
14	NaOH	0·16	none	—	very few	—

Comments on Series II.

Germination took place after 42 hours in all the solutions with the exception of 0·16 per cent. NaOH and 0·16 per cent. "Polysulphides K." These showed, however, three or four suspects after 88 hours. Only a few had germinated in 88 hours in 0·16 per cent. "Polysulphides S" and 0·16 per cent. Sample *A*.

After remaining 42 hours in the four solutions given below the spores were transferred to pure water. Germination of these took place in each case in 46 hours, showing that 0·16 per cent. solutions of these substances are not strong enough to prevent germination.

SERIES II (*continued*).

42 hours in the Solutions, and then into Pure Water.

No.	42 hours in	Strength per cent.	25 hours in water		46 hours in water	
			Number germinated	Length in spore diam.	Number germinated	Length in spore diam.
22	Polysulphides S	0·16	few		nearly all	
20	Polysulphides K	0·16	none		very few	
18	Sample *A*	0·16	few		nearly all	2
14	NaOH	0·16	some	6	,, ,,	long

As a check upon the trials of Series I, fresh trials were made with 0·33 per cent. solutions, and the results are recorded in Series III.

SERIES III. *Fresh trial of* 0·33 *per cent. Solutions.*

Particulars of Solutions			Behaviour of spores of *Botrytis cinerea*			
			25 hours		46 hours	
No.	Composition	Strength per cent.	Number germinated	Length of hyphae in spore diam.	Number germinated	Length of hyphae in spore diam.
1	Na_2S	0·33	none		very few	
2	KHS	,,	,,		none	
3	KOH	,,	,,		,,	
4	NaOH	,,	,,		,,	
5	Sample *B*	,,	,,		none *	
6	Sample *A*	,,	,,		nearly all	1½
7	Polysulphides K	,,	,,		none	
8	Polysulphides S	,,	,,		,,	
9	$Na_2S_2O_3$	,,	some	4	many	6
10	Na_2SO_3	,,	very few		,,	2
11	$Ca(OH)_2$	,,	many	4—5	nearly all	10

* Insufficient inoculation.

Comments on Series III.

Spores failed to germinate in 0·33 per cent. NaOH, KOH, KHS, "Polysulphides K" and "Polysulphides S" after 46 hours.

The experiments recorded in Series II indicated that 0·16 per cent. solutions of NaOH and "Polysulphides K" were nearly strong enough to prevent germination. As 0·33 per cent. solutions had already proved effective, it was decided to try the effect of 0·25 per cent. solutions, these being intermediate in strength. The results are given in Series IV.

SERIES IV.

Particulars of Solutions			Behaviour of spores of *Botrytis cinerea*	
			140 hours	
No.	Composition	Strength per cent.	Number germinated	Length of hyphae in spore diameter
1	Na_2S	0·16	all	
2	,,	0·25	,,	
3	,,	0·33	,,	
4	KHS	0·16	nearly all	
5	KOH	0·33	none	
6	NaOH	0·16	very few	1
7	,,	0·25	none	
8	,,	0·33	,,	
9	Sample *A*	0·16	,,	
10	,,	0·25	,,	
11	Polysulphides K	0·16	many	1
12	,,	0·25	none	
13	Polysulphides S	0·16	many	1
14	,,	0·25	none	

Comments on Series IV.

It will be noted that 0·16 per cent. solutions of NaOH, "Polysulphides K," "Polysulphides S," and Sample *A*, appear on the border line of prevention of germination, and that 0·25 per cent. solutions of these substances have proved effective.

The next series of trials was designed with a view to determining the length of time necessary, not only to prevent germination, but to kill the spores outright. The solutions were therefore placed in stoppered bottles, and a large number of spores introduced into each. The bottles were shaken frequently so as to insure contact of the solutions with every spore, and after periods of one, two, three, four, five, and six hours, a loopful of the liquid containing a fair quantity of

spores was introduced into water contained in watch-glasses, and incubated for 78 hours. The results are shown in the following table.

SERIES V.

Particulars of Solutions			Number germinating when transferred to pure water after exposure to the solutions for different lengths of time						
			Hours						
No.	Composition	Strength per cent.	1	2	3	4	5	6	27
1	Sample *A*	0·33	all	all	all	all	all	all	none
2	Polysulphs K	0·33	,,	,,	,,	,,	,,	,,	,,
3	Polysulphs S	0·33	,,	,,	,,	,,	,,	,,	,,
4	KOH	0·5	,,	,,	many	very few	none	none	,,
5	NaOH	0·5	many	none	none	none	,,	,,	,,
6	Na_2S	0·5	all	all	all	all	all	all	,,
7	KHS	0·5	,,	,,	,,	,,	,,	,,	,,

The microscope in all cases revealed a considerable number of spores in the water. The soda and potash tests were incubated for a further 23 hours, making 101 hours in all, but no germination could be seen.

Comments on Series V.

The deadly effect of alkalies is clearly shown, two hours in 0·5 per cent. NaOH and three to four hours in 0·5 per cent. KOH being sufficient to kill all spores of Botrytis: 6 to 27 hours were required in the other solutions.

Adopting the same method as that used in Series V, further trials were made with soda and potash to determine the length of exposure necessary to kill the spores of Botrytis in solutions weaker than 0·5 per cent. The results are shown in Series VI.

SERIES VI. *Weaker Solutions.*

Final results after 8 days in water.

Particulars of Solutions			Number germinating when transferred to pure water after exposure to the solutions for the following lengths of time					
No.	Composition	Strength per cent.	1 hour	2 hours	3 hours	4 hours	5½ hours	6 hours
1	KOH	0·25	all	all	all	all	all	all
2	,,	0·33	,,	?	?	few	none	none
3	,,	0·5	,,	all	nearly all	none	,,	,,
4	NaOH	0·25	many	very few	none	,,	,,	,,
5	,,	0·33	few	nearly all	,,	,,	,,	,,
6	,,	0·5	nearly all	none	,,	,,	,,	,,

Comments on Series VI.

The results agree fairly well with those given for soda and potash in Series V, soda again showing a higher toxicity than potash.

The next series of experiments was conducted with a view to imitating the action of sprays upon the leaves of plants when the weather is not too dry, and with no wind to assist evaporation. It was argued that as each droplet of spray evaporates, the solution gets nearer to saturation. Two loops of each liquid were placed in a watch-glass, and inoculated with spores which were stirred into the liquid as much as possible, the aim being to get them all wetted with the solutions. These watch-glasses were allowed to stand under inverted pans until the liquid had completely evaporated to dryness. About an hour was taken before dryness was reached. The watch-glasses were next half filled with water, and incubated at 22° C. The results are given in Series VII.

SERIES VII.

Particulars of Solutions			Separate drops of the solutions inoculated with spores of *Botrytis cinerea*, and allowed slowly to evaporate to dryness in still air. Water afterwards added and incubated at 22° C.			
			On the top		Deep in the liquid	
No.	Composition	Strength per cent.	From single spores	Germinated from clusters	Long thin filaments ? Botrytis	Germinated from clusters
1	Distilled water	—	all			
2	Na_2S	0·16	nearly all			
3	,,	0·25	very few		very few	
4	KHS	0·16	many		many	
5	,,	0·25	some	many	,,	
6	KOH	0·08	none	few		three
7	,,	0·16	,,			one
8	,,	0·25	,,			two
9	NaOH	0·08	,,		few	
10	,,	0·16	,,		few	
11	,,	0·25	,,			few
12	Sample *B*	0·25	,,	two	few	
13	,,	0·33	,,	five	two	
14	Sample *A*	0·16	?		many	
15	,,	0·25	?		,,	
16	$Na_2S_2O_3$	0·16	one		one	
17	,,	0·25	few		many	
18	Na_2SO_3	0·16	few		few	
19	,,	0·25	few		,,	
20	Polysulphides K	0·16	none	two	,,	
21	,,	0·25	,,		very few	
22	Polysulphides S	0·16	,,	three	few	
23	,,	0·25	,,		very few	

Comments on Series VII.

With regard to the long hyphae in the depth, in no case could these be traced to a single spore. They appeared to have a much smaller diameter than a normal hypha of Botrytis. In a few cases, however, a normal Botrytis hypha was traced back to a spore in a cluster of spores. It was evident that some of the spores in the cluster were protected from contact with the solutions before they had evaporated. The remarks in the first column concern single spores quite detached from the clusters, except where stated. Unfortunately, the writer neglected to stir after putting water into the watch-glasses, otherwise all the spores would have risen to the surface. Another method must be devised for ensuring that all the spores are wetted by the solutions as they get more concentrated, but one is then confronted with the question: Could such a method imitate the effect of sprays on the plant? All we can say from the above results is that in the cases of soda and potash especially, practically all the single spores are killed as the solutions get more concentrated. In all the watch-glasses except where stated a quantity of spores were revealed by the microscope, and each watch-glass was subjected to a prolonged examination until the writer was satisfied that no cases of germination had been overlooked.

Trials with Spores of Gooseberry Mildew.

Attempts were made to repeat these experiments with spores of American Gooseberry Mildew. Spores of this fungus were therefore obtained with the object of conducting series of trials similar to those carried out with spores of Botrytis. At the very outset, however, a great difficulty was encountered. In spite of every persuasion, only here and there could a spore be induced to germinate even in pure water. These isolated cases were always found to be the slightly bigger spores found at the extreme ends of normal chains of conidia. The end spore is always more vigorous than the others in the chain. Many trials were made with spores of different age in various media, and incubated at different temperatures, but with no satisfactory results. Making the media slightly alkaline, or slightly acid with citric acid, also failed to produce any effect. Large numbers of spores were introduced into each solution. The trials were not persevered with owing to this unsatisfactory behaviour. The best results, however, are given below in Series VIII.

SERIES VIII.

Trials with Spores of American Gooseberry Mildew.

Particulars of Solutions			Behaviour of spores of American Gooseberry Mildew		
			160 hours at 20° C., but no further growth after 88 hours in any case		
No.	Composition	Strength per cent.	Number germinated	Length of hyphae in spore diam.	Number of spores visible under the microscope
1	Distilled water	—	few	4	small quantity
2	Na_2S	0·08	four		large number
3	,,	0·16	few	long	,, ,,
4	KHS	0·08	few	4	,, ,,
5	,,	0·16	one		few
6	KOH	0·08	few		large number
7	,,	0·16	none		,, ,,
8	NaOH	0·08	fourteen	10	,, ,,
9	,,	0·16	none		,, ,,
10	Sample *B*	0·08	one		few
11	,,	0·16	two?		large number
12	Sample *A*	0·08	none		,, ,,
13	,,	0·16	one?		,, ,,
14	Polysulphides K	0·08	one		few
15	,,	0·16	few	long	large number
16	Polysulphides S	0·08	two?		
17	,,	0·16	none		large number
18	$Na_2S_2O_3$	0·08	six	4	small quantity
19	Na_2SO_3	0·08	few	long	large number
20	,,	0·16	,,	,,	,, ,,

Comments on Series VIII.

It will be observed that the effect of the solutions upon the spores of American Gooseberry Mildew is similar in general trend to the effect produced upon the spores of Botrytis.

Summary and Conclusions.

The term "Liver of Sulphur" does not represent a standard product. Samples from different sources show variation in solubility, alkalinity, and content of potash, sodium carbonate being sometimes used in its manufacture instead of the potassium compound.

Liver of sulphur contains various oxidation products in addition to sulph-hydrate, sulphide and polysulphides of either potassium or sodium, and free sulphur is present in the majority of cases.

The oxidation products present possess little or no fungicidal properties when tried separately in weak solutions upon the spores of *Botrytis cinerea*. Saturated sulphuretted hydrogen solution and free sulphur have absolutely no adverse effect upon the germination of spores of this fungus.

0·16 per cent. solutions of soda, Polysulphides K, Polysulphides S, and Sample *A* were not quite strong enough to prevent germination, but 0·25 per cent. solutions of these substances were effective for this purpose.

The spores of Botrytis were killed after an exposure of 1—2 hours to 0·5 and 2—3 hours to 0·25 and 0·33 per cent. soda. 0·5 per cent. potash required 3—4 hours and 0·33 per cent. 4—5½ hours. 0·33 per cent. solutions of Polysulphides K, Polysulphides S, and Sample *A* required somewhere between 6 and 27 hours' exposure.

These results point to the conclusion that the free alkali soda is the most potent fungicidal agent in the whole mixture, potassium hydroxide being also poisonous, but to a smaller extent. The experiments of Series VII bear out this statement. The use of soda instead of potash increases therefore the value of liver of sulphur as a fungicide, and this should bring about a considerable reduction in price.

The presence of air throughout the experiments assisted the germination of the spores.

Roughly speaking, the effect of the solutions upon the spores of American Gooseberry Mildew was similar in general trend to the effect upon spores of Botrytis. The spores of this fungus, however, would not germinate satisfactorily in the laboratory under any circumstances.

Throughout the experiments the importance of thoroughly wetting the spores with the solutions is emphasized, and it is doubtful whether one spraying is ever sufficient to do this, and to check a disease.

Relative value of Free Alkali and the Sulphur as Polysulphide.

The hydrolytic dissociation of the polysulphides in dilute solution prevents their effect upon spores from being determined independent of the free alkali. Absorption of free alkali by the spores would, by its removal from the sphere of action, result in a disturbance of the equilibrium, and this would bring about further dissociation and provide further available alkali.

A considerable proportion of the potassium content could therefore be yielded as free alkali, provided the absorption was continuous.

Calculated upon the content of potassium and sulphur (30·6 per cent. K and 44·7 per cent. S) in the original polysulphide mixture, the percentages of sulphur and potassium, and the amount of free potash that could be yielded on continuous hydrolytic dissociation accompanied by absorption are given in Table IV.

TABLE IV.

No.	Solution	Strength per cent.	S per cent.	K per cent.	K calculated as KOH
1	Polysulphides K %	0·16	0·24	0·16	0·23
2	,, S %	0·16	0·16	0·11	0·15
3	,, K %	0·25	0·37	0·25	0·36
4	,, S %	0·25	0·25	0·17	0·24

Spores of Botrytis are prevented from germinating in 0·16 per cent. solutions of potash and in No. 1 to about the same extent, although, it will be noted, solution No. 1 would yield one and a half times as much free alkali as is contained in 0·16 per cent. potash, assuming that the whole of the potassium of No. 1 could be yielded as free alkali. Again solution No. 3 and 0·25 per cent. potash are both just strong enough to prevent germination, and the latter kills the spores outright in less time, in spite of the fact that solution No. 3 would be capable of yielding more than double the amount of free potash on hydrolysis, if accompanied by continuous absorption of the alkali. Solution No. 3, however, also contains 0·37 per cent. of sulphur as Polysulphides, etc. If any effect is to be attributed to the sulphur, its conjoint action with the free alkali should invest the polysulphides mixture with a much higher toxicity than the alkali, but that the reverse is the case will be evident if a study be made of the results of the experiments recorded in Series V, VI, and VII. The argument could be made even more forcible by considering soda instead of potash.

The above argument includes no consideration of the mechanical action of free sulphur as a possible fungicidal factor. When plants are sprayed with weak solutions of liver of sulphur, a very fine precipitate of free sulphur is deposited on the leaves in a very short time. The majority of samples also contain a certain amount of free sulphur. Finely powdered sulphur scattered on the leaves of plants is said to be a check upon certain mildews, but it is suggested that ordinary dust is equally efficacious. This must be a subject for further investigation.

From the foregoing considerations it appears highly probable that solutions of soda, much weaker than the solutions of liver of sulphur usually sprayed, would be equally as effective as the latter, and the cost of spraying greatly reduced. Spraying tests must first be made, however, to determine whether the free alkali injures the leaves, and this will be investigated in the field as soon as possible. Should injury to the leaves result, then similar solutions of sodium or potassium carbonate will be tried, as these would hydrolyse to about the same extent as the polysulphides and sulphides in the liver of sulphur, preventing too high a concentration of free alkali. This suggestion involves the assumption that no fungicidal value can be attached to the mechanical effect of the free sulphur deposited on the leaves of plants when liver of sulphur is used.

Proposed Method of Analysis of Commercial Liver of Sulphur.

1. *Potash.* Obtain a rough idea of the content of potash with sodium cobaltinitrite solution. If the sample contains little potash, this should be estimated by the method used on page 403.

2. *Free Alkali and Free Sulphur.* Weigh out 1 gm., dissolve in water, filter through tared filter papers into a 200 c.c. flask, wash, dry, and weigh the insoluble free sulphur. Make the solution up to the mark. Take 20 c.c., add 50 c.c. distilled water, and run in $\frac{N}{10}$ acid with constant vigorous stirring until a faint opalescence appears. A point will be reached when one or two drops will very perceptibly accentuate the opalescence.

3. *Total Alkali, assuming complete dissociation.* Take 20 c.c. of the above solution, add 3 c.c. normal acid from a burette and 50 c.c. of distilled water. Evaporate on the water bath to about 20 c.c. Filter and wash thoroughly with cold water. Cool and titrate with $\frac{N}{10}$ soda with methyl orange as indicator. The following figures were thus obtained for Sample *A*:

20 c.c. solution taken = 0·1 gm. sample,

10·85 c.c. $\frac{N}{10}$ soda run in,

1 c.c. = ·0047 gm. K_2O,

10·85 c.c. = ·0510 gm. K_2O

= 51·0 per cent. K_2O (equal to 60·77 per cent. KOH).

By the sodium cobaltinitrite method given on page 403, 51·3 per cent. K_2O was found.

Precipitated Sulphur.

Take 100 c.c. of the solution, add 10 c.c. normal acid and heat on the water bath until the sulphur collects together at the bottom. Filter on tared papers, wash, dry, and weigh.

Should the sample contain soda, the amount present can be calculated from the titration after making allowance for potash estimated by the cobaltinitrite method.

In the light of the inferences made on page 414, and assuming free sulphur to possess mechanical effect as a fungicidal agent, the above method of analysis should supply all the information desired, and it possesses the merit of being easily carried out.

THE INTERPRETATION OF EXPERIMENTAL RESULTS.

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AND

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THE interpretation of experimental results of any kind requires considerable caution, and this is more than ordinarily necessary in the case of agricultural experiments. The chemist deals for the most part with chemical compounds for which there are well recognised criteria of purity. He can make use of absolute methods of analysis, and is usually free from worries about sampling. But the agriculturist must often use methods which are at the best only conventional: he deals with substances of variable composition such as soils, manures, and crops, where errors of sampling are always apt to occur: he must at times trust to weighings or measurements of animals whose individuality is even more marked than that of plants.

Everyone who has carried out experiments in the field or in the farmyard, must be well aware that the result of a single experiment is very often entirely misleading. Yet it is still a common practice to publish single results and to base practical advice upon them. With the great growth of interest among the farming community and the increasing tendency of the farmers to take note of the work of the experimentalist and to act upon it, it is becoming increasingly important that due caution should be exercised by experimenters in interpreting their results before laying them before the agricultural public. It is for these reasons that the preparation of the following account of some of the statistical methods, which may be used for the investigation of the accuracy of experimental results, has been undertaken.

Before proceeding to discuss the subject in detail, it may be well to consider the kind of problems which face the experimenter who wishes

justly to interpret his results. The commonest problem is presented by the well-known discordance which is always found between a number of separate determinations of the value of the same quantity. In this case two things are required. The best value of the several determinations must first be found. This is usually done by taking the average, *i.e.* by finding the arithmetic mean. Here the question arises: are the results such that averaging is justifiable, and, if so, is it possible to form any kind of estimate of the reliability of the average?

Again, it frequently happens that the experimenter finds it necessary to compare the results of two systems of manuring or feeding. Before he can do so with any real certainty of the accuracy of his verdict, he must know that the differences he observes between his field plots, or his lots of animals, are considerably greater than the error involved in his methods of experimenting. In other words he must have a definite idea of the degree of accuracy of his method.

The average or arithmetic mean.

Some years ago, one of the authors of this paper was engaged in an investigation which involved the analysis of very large numbers of individual roots. Among other things the percentage of dry matter was determined in each of 160 individual roots of a strain of Golden Globe mangel. The lowest result was 10·7 per cent., the highest 19·7, the average or arithmetic mean of all the 160 results 14·5 per cent. Is it justifiable to take an average of results varying so widely as these, and, if so, what is its degree of accuracy? Consider why there should be any variation in content of dry matter in roots of the same strain grown side by side and sampled and analysed in the same manner. In the first place mangels are very easily cross fertilized, and no commercial strain will be a pure line, that is to say, descended from a single parent. Varying parentage will no doubt be one cause of variation, and it is equally likely to make any individual root better or worse than the average. Again, the soil, and consequently the food supply, varies from place to place; the roots are not hoed out with mathematical accuracy; the distribution of manure is never absolutely uniform; and even the most careful analyst is not proof against experimental error. These are some of the causes of the variations observed in the results of dry matter determinations in individual roots, and so far as can be seen, each separate cause is equally likely to make the percentage of dry matter in any individual root come out higher or lower than the

average. In the great majority of cases it is to be expected that about half the causes will tend to produce a high result, and will be balanced by the other half acting in the opposite direction. The majority of the results therefore should come out fairly near the average. In some cases, however, more of the causes will tend one way than the other. Whenever this is so the result will differ considerably from the average. In a few cases nearly all, and perhaps in very rare cases all, the causes of variation will tend one way, and very large divergencies from the average will be produced. We should expect, therefore, that in a large number of determinations like those under discussion, many results would come out near the average, and that the number of results diverging from the average in each direction would be fewer the further the divergence from the average.

Frequency Curves.

This is well shown by plotting the results in the form of a frequency curve, a study of which will often indicate at once whether the results in question can be fairly averaged. In plotting such a curve the results are first classified. In the present case convenient classes are made by taking one per cent. intervals from 10 to 20. These are marked off along the horizontal axis. The numbers of results falling in each class are marked off along the vertical axis by placing a dot for each result at equal intervals along vertical lines corresponding to the middle value of each class.

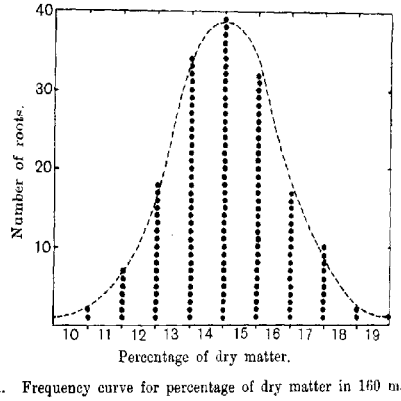

FIG. 1. Frequency curve for percentage of dry matter in 160 mangels.

Inspection of the curve produced by joining the highest dots in each column shows at once that the results in question are quite in accordance with expectation. A large number of results are found to come out very near the average, and the numbers fall away symmetrically on each side as the divergence from the average increases. The symmetry is less marked when the numbers become very small, which is also quite in accordance with expectation. It seems fair to conclude that in the individual roots examined the variation is quite normal, and it is therefore justifiable to average the results.

The device of plotting a frequency curve can be employed to study the variability of almost any kind of quantity. In Fig. 1 it was employed for a number of analyses of mangels. In Fig. 2 the live weight increases of 100 sheep all fed on a similar diet are plotted.

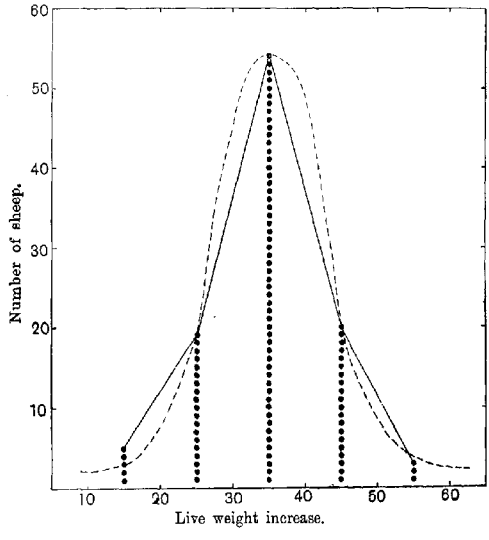

FIG. 2. Live weight increases of 100 sheep.

The curve is very similar to Fig. 1. Most of the results are near the average and the numbers fall off with increasing rapidity as the results get further from the average. Again it is fair to conclude that the variation is quite normal, and it is therefore legitimate to average the results.

It does not always happen, however, that a frequency curve has the symmetrical form shown in Figs. 1 and 2. Sometimes the departure from the normal symmetrical form is very striking, and it will be instructive to study one or two such instances. The curve in Fig. 3 is

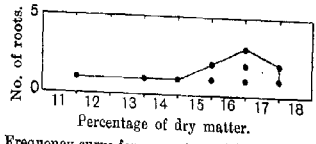

FIG. 3. Frequency curve for percentage of dry matter in 10 roots.

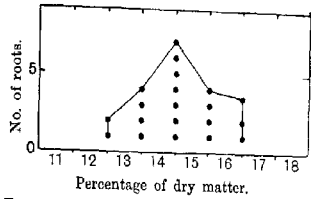

FIG. 4. Frequency curve for percentage of dry matter in 20 roots.

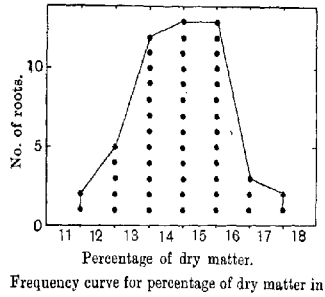

FIG. 5. Frequency curve for percentage of dry matter in 50 roots.

altogether unsymmetrical. Ten results are not enough to give the laws of chance a fair opportunity of asserting themselves. In Fig. 4 the curve for 20 results is very nearly symmetrical, but this may be accidental, for Fig. 5, in which the results for 50 roots are plotted, is still lop-sided. Lack of symmetry in a frequency curve plotted from small numbers of results does not necessarily mean that there is anything abnormal. The laws of chance cannot be expected to give normal results with small numbers. Consequently the average of a small number of observations is not reliable.

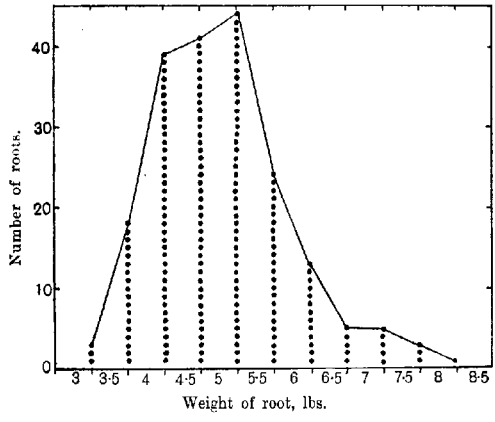

FIG. 6. Weights of 196 roots.

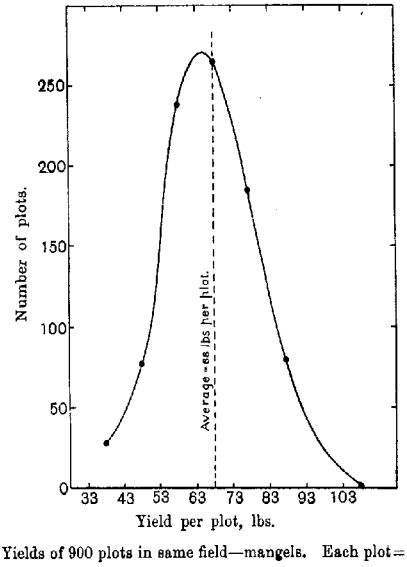

FIG. 7. Yields of 900 plots in same field—mangels. Each plot = $\frac{1}{1000}$ acre.

This explanation, however, cannot account for the skewness of the curve shown in Fig. 6. Here the number is quite large enough to give a symmetrical curve, and the failure to obtain one must be accounted for. It will be noticed that the curve is much too steep on the small root side. This indicates that some cause is at work which either makes an abnormally high proportion of large roots, or unduly decreases the number of small roots. No doubt the latter is the correct explanation, for very weakly plants which would produce very small roots are destroyed in the processes of hoeing and singling. Another possible explanation is that in sampling, the very small roots were unconsciously passed over.

Fig. 7 represents the frequency curve obtained by plotting the results of weighing 900 plots of mangels each $\frac{1}{1000}$ acre in area, forming part of an apparently uniform crop. The numbers here are amply sufficient to give a really symmetrical curve. The curve is, however, distinctly unsymmetrical. The average is not coincident with the highest point of the curve, which falls away much more steeply on the low weight side. This indicates that there is present some cause of variation of crop which gives the results a distinct bias towards the high side. Further examination of the results shows that the depth of soil is not uniform and this fact is largely responsible for the skewness of the curve.

In Fig. 8 a more interesting type of abnormal frequency curve is shown. It gives the results of plotting the lengths of the glumes of 595 individual wheat plants. A glance suggests that the plants do not belong to one strain and that the results are certainly not suitable for averaging. The plants in question were the second generation from a cross between Rivet wheat with an average glume length of 9 mm., and Polish wheat with an average glume length of 28 mm. The curve shows clearly that the plants examined are segregated into three groups, one resembling the short glumed Rivet parent, one like the long glumed Polish parent, and the third intermediate between these, with an average glume length of 17 mm. This example shows well the use of the frequency curve. The shape of the curve shows clearly that the material under examination is heterogeneous, and the results of the measurements are therefore unsuitable for averaging.

The above instances are enough to illustrate the uses of the frequency curve. By plotting such a curve it is possible to get a definite idea as to the homogeneity of the material under investigation, whether it consists of analyses of individual plants, measurements of

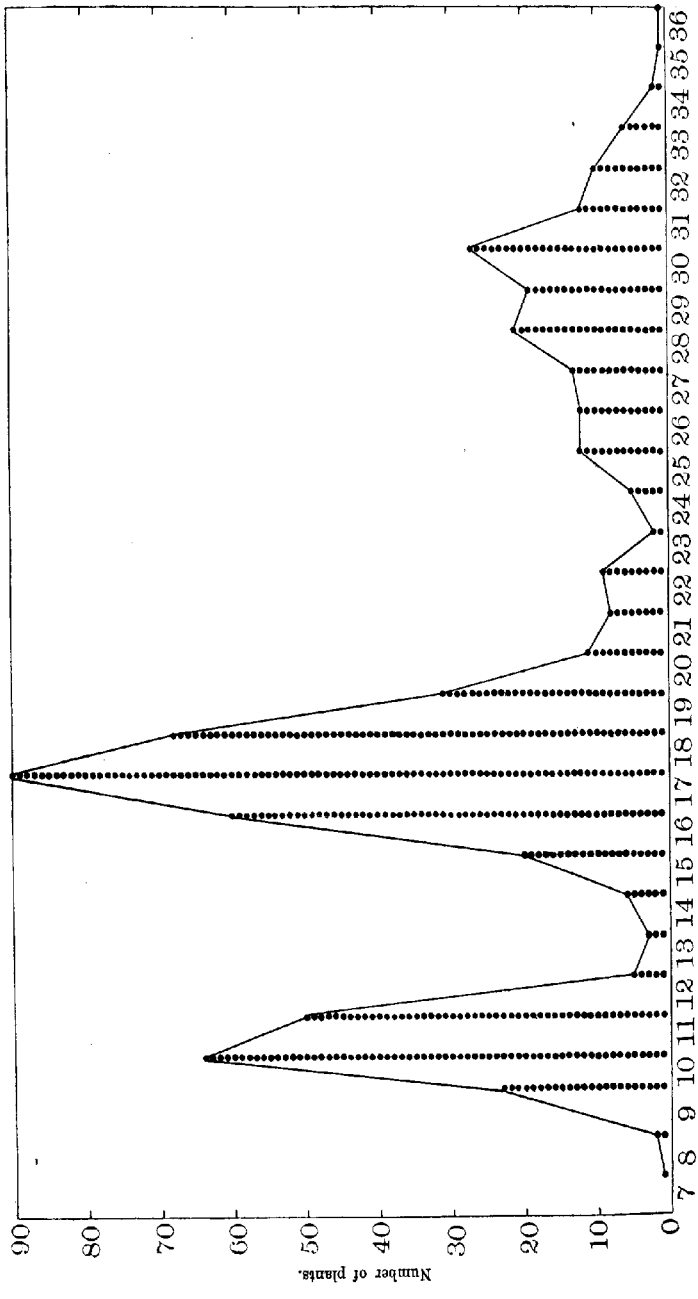

FIG. 8. Frequency curve for 595 hybrid wheat plants.

animals, or weights of field plots. The frequency curve shows if the results can properly be averaged, or if they are vitiated by lack of homogeneity of the material, or by a systematic error due to variation in soil or bias in sampling. It should be added that a skew curve does not always indicate that the material under investigation is heterogeneous. In some cases where the observations are biological in nature, skew curves are met with which can only be explained by assuming that the conditions of growth of the plants or animals on which the observations were made give a bias in one or the other direction. In many such cases, *e.g.* Figs. 6 and 7, the average will be fairly reliable if the number of observations is large enough.

Probable Error.

It might seem at first that no two branches of study could be more widely separated than Agriculture and Astronomy. A moment's consideration, however, will show that they have one point in common: both are at the mercy of the weather. The astronomer's measurements come short of absolute accuracy because of a great number of varying atmospheric conditions, each of which is equally likely to make any one result high or low. He has to obviate this unavoidable lack of accuracy by making many independent observations, and taking their average. This is, or should be, the method followed by the agriculturist.

The astronomer, being a mathematician, has devised a method of estimating the accuracy of his averages, which he invariably applies with great advantage. The agriculturist cannot do better than follow his example. By doing so he will often be prevented from publishing experimental results which can only be misleading to those who read and act on them. The method consists in finding the "probable error" of a result by the device known as "least squares." It is not necessary here to expound the mathematical basis of "least squares." A few concrete examples will probably be more to the point. The method of working is illustrated in the following table, in which the probable error is calculated for determinations of dry matter in ten individual mangel roots.

The first column gives the percentage of dry matter in each root. In the second column are the differences, d, of each result from the mean. The third column contains the squares of these differences, d^2. Below are given the average of the ten percentages, and the sum of the squares of the differences, Σd^2.

TABLE I.

Percentage of dry matter	d	d^2
14·2	1·2	1·44
16·6	1·2	1·44
13·8	1·6	2·56
11·3	4·1	16·81
17·5	2·1	4·41
15·0	0·4	0·16
16·8	1·4	1·96
17·2	1·8	3·24
15·1	0·3	0·09
16·6	1·2	1·44
Average 15·4		$\Sigma d^2 = 33{\cdot}55$

The probable error is then found from the formula—probable error of any one result,

$$\text{p.e.} = 0{\cdot}67\sqrt{\frac{\Sigma d^2}{n-1}},$$

where n is the number of results, in this case ten. Thus:

$$\text{p.e.} = 0{\cdot}67\sqrt{\frac{\Sigma d^2}{n-1}}$$

$$= 0{\cdot}67\sqrt{\frac{33{\cdot}55}{9}}$$

$$= \pm 1{\cdot}3.$$

The probable error thus determined is a measure of the reliability of any one result. It is such that taking any single result at random, the chances are even for or against that result differing from the average by the amount of the probable error. In other words half of the results should differ from the mean by less than the probable error, the other half by more. Inspection of column d shows that in the case under discussion this is so. This does not always happen when dealing with small numbers, for the same reason that small numbers do not usually give symmetrical frequency curves. For this reason too the probable error calculated from a small number of observations or measurements is not usually very reliable. This is well shown in the following table which refers to the same series of observations used for plotting the frequency curves shown in Figs. 1, 3, 4 and 5.

Table II.

No. of roots	Probable error of result	Cp. curve
10	±1·3	Fig. 3
20	±0·8	Fig. 4
50	±0·9	Fig. 5
160	±1·1	Fig. 1

With 160 roots a very symmetrical and smooth curve is obtained, Fig. 1, which shows that the number is sufficient to give the laws of chance fair play. Doubtless, therefore, the true probable error involved in sampling and analysing roots as described is ± 1·1 for any one result. The variations of the probable error calculated from the smaller numbers must be taken with the lack of symmetry of their frequency curves, Figs. 3, 4 and 5, as evidence that small numbers of roots do not give fair samples.

Frequency curve and probable error.

The frequency curve is closely connected with the probable error as determined by the least square method. This is shown clearly in Fig. 9,

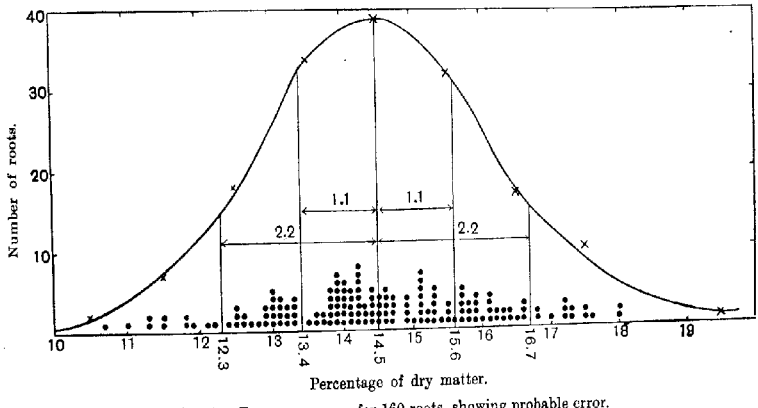

Fig. 9. Frequency curve for 160 roots, showing probable error.

which is the frequency curve for the percentage of dry matter in 160 roots, the same curve as Fig. 1, but with the horizontal scale doubled. This makes it possible to put the dot representing the percentage of

dry matter in each root in its own proper position instead of at the middle point of its class. The dots falling inside each class, *i.e.* 10—11 per cent., 11—12 per cent. and so on, are then counted, a cross put on the middle line of each class to represent the number of individuals in the class, and the curve drawn through these crosses.

A vertical line is then drawn through the highest point of the curve which in such a symmetrical curve coincides with the mean. Other vertical lines are drawn at distances of 1·1 per cent. on each side of this, *i.e.* at 13·4 per cent. and 15·6 per cent. Since 1·1 per cent. is the probable error of one analysis, it is possible by counting the dots inside and outside these two lines to check the definition of probable error. By the definition as many results should fall inside as outside. The numbers are, inside 81 dots, outside 38 on the low side, 41 on the high side, total 79. These are near enough to the theoretical figures of 80 and 80, and it seems to be quite clear that the least square method gives a probable error which satisfies the definition that half the results differ from the mean by less and half by more than the probable error. Two more vertical lines are drawn in the figure at distances corresponding to twice the probable error, *i.e.* 2·2 per cent. on each side of the mean. By counting the dots inside and outside these lines we find that there are 25 dots outside and 135 dots inside, or only 25 results differ from the average by more than twice the probable error whilst 135 results differ by less than that amount. The odds against any one result differing from the average by more than twice the probable error are therefore 135 to 25, or 5·4 to 1. Since a symmetrical frequency curve is a well-known mathematical curve expressed by an equation, it is possible to calculate the area inside and outside lines drawn vertically through any point, as for instance the lines in question. Now the area inside these two lines calculated in this way is $4\frac{1}{2}$ times greater than the area outside. Also the area should by theory measure the number of dots. Hence the calculated odds against any one result differing from the average by more than twice the probable error is $4\frac{1}{2}$ to 1. This again is as near as can be expected to the observed number of 5·4 to 1, considering that the numbers, only 25, are really very small to give the laws of chance fair play.

The agreement in the two above cases is quite good enough to show that the odds calculated theoretically from the equation for the frequency curve fairly represent what actually occurs in practice. These odds are given in the following table:

TABLE III.

Difference from mean in terms of probable error	Odds against such difference being due to normal variation
1·00	1 to 1
1·25	3 ,, 2
1·44	2 ,, 1
1·71	3 ,, 1
1·90	4 ,, 1
2·00	9 ,, 2
2·05	5 ,, 1
2·50	10 ,, 1
2·93	20 ,, 1
3·00	22 ,, 1
3·20	30 ,, 1
4·00	140 ,, 1
4·90	1000 ,, 1
5·00	1350 ,, 1

Probable error of average.

To find the probable error of an average, the formula used is

p.e. of average of n results,

$$\text{or } (\text{p.e.})_n = 0{\cdot}67\sqrt{\frac{\Sigma d^2}{n(n-1)}}.$$

Comparing this with the previous formula it is clear that the probable error of the average can be found at once by dividing the probable error of one result by the square root of the number of results averaged. The following table gives the probable errors of the average of 10, 20, 50, 100, and 160 determinations of dry matter in individual roots.

TABLE IV.

No. of results, n	P.e. of 1 / $\sqrt{n}$	P.e. of average of n results
10	$1{\cdot}1/\sqrt{10}$	0·35
20	$1{\cdot}1/\sqrt{20}$	0·24
50	$1{\cdot}1/\sqrt{50}$	0·16
100	$1{\cdot}1/\sqrt{100}$	0·11
160	$1{\cdot}1/\sqrt{160}$	0·09

The probable error of the average decreases as the number of results averaged increases, or to be more precise, the accuracy of the

average increases with the square root of the number of observations. By taking a large enough number of observations the precision can be increased to any desired extent, except that the observations may all be subject to a systematic error.

This fact can be utilized in the following manner. In a series of field experiments carried out in the Eastern Counties with the object of finding the effect of manuring on the composition of the mangel crop, the following results were obtained. The experiments were carried out at five stations, and 100 roots were sampled at each station for each plot. The figures given therefore represent the average of the analysis of 500 roots.

TABLE V.

Manuring	Average percentage of dry matter
Unmanured	14·4
Complete artificial manure	13·6
Farmyard manure	12·6
Farmyard manure + complete artificials	12·2

Now the probable error of the average of the analysis of 500 roots will be $\frac{\text{p.e. of 1}}{\sqrt{500}} = \frac{1{\cdot}1}{\sqrt{500}} = \pm 0{\cdot}05$ per cent. In a comparison of two results, each of which is liable to this error, their difference will be liable to a greater error. In fact the probable error of the difference between two results is $\sqrt{2} \times$ p.e. of 1 result, *i.e.*, p.e. of the difference between the means of 2 sets of 500 roots is $\pm 0{\cdot}05 \times \sqrt{2} = 0{\cdot}07$ per cent. The difference in percentage of dry matter between the roots manured with artificial manure and the unmanured roots is $14{\cdot}4 - 13{\cdot}6$ per cent. $= 0{\cdot}8$ per cent., and this difference is 11 times the probable error of the average 0·07 per cent. Again the difference between the percentages of dry matter in the roots manured with farmyard manure alone and those getting farmyard manure and artificials is 0·4 per cent., which is 6 times the probable error. From the definition of probable error it follows that a difference of about the same magnitude as the probable error is just as likely as not to be due to normal variation. Such a difference therefore cannot be taken to be really significant. For instance, if the difference between the average analyses of the differently manured plots had been only 0·07 per cent. or thereabouts, it would have been fair to conclude that manuring had no influence on the percentage of dry matter. But if the difference is greater than

the probable error, the chance that it is due only to normal variation rapidly decreases.

In this case the odds given in Table III do not apply, for the difference to be measured is definitely a *decrease* in percentage of dry matter, *i.e.* in one direction only. The variations produced will therefore be all on one side of the average, the low side, and the odds must be calculated from the ratio of the area of the piece of curve outside the vertical line drawn at a given distance on the low side of the average.

For instance, in Fig. 9, the odds against any one result being *less than the average* by more than the probable error will evidently be the ratio of the whole of the area to the right of the line drawn 1·1 below the average to the area of the small piece to the left of that line. This ratio is clearly 3 to 1. Hence the odds against any one result being *less* than the mean by a quantity more than the probable error are 3 to 1. Counting the dots, there are found to be 122 to 38, or 3·2 to 1. Again the calculated and observed results are near enough to justify the application of the least square method.

The following table gives the odds against any one result differing from the mean *in one direction* only by more than any given number of times the probable error. Also the odds for the difference in one direction only between two results each affected by the same probable error.

TABLE VI.

Difference from mean in terms of probable error of one individual	Difference between two results in terms of probable error of each result	Odds against such difference being due to normal variation
1·00	1·41	3 to 1
1·25	1·76	4 ,, 1
1·44	2·03	5 ,, 1
1·58	2·23	6 ,, 1
1·71	2·41	7 ,, 1
1·81	2·55	8 ,, 1
1·90	2·68	9 ,, 1
2·00	2·83	10 ,, 1
2·48	3·50	20 ,, 1
2·70	3·81	30 ,, 1
2·89	4·07	40 ,, 1
3·00	4·24	44 ,, 1
3·03	4·28	50 ,, 1
3·44	4·85	100 ,, 1
4·00	5·66	290 ,, 1
5·00	7·07	2700 ,, 1

Reference to the above table shows that the odds are very heavy against a *decrease* of 11, or even 6 times, the probable error being

due to normal variation. It is therefore fair to conclude that the differences observed between the percentages of dry matter in the experiments under discussion are practically certain to be real significant differences produced, not by normal variational causes, but by the different treatment of the plots, *i.e.* by manuring.

Probable error of feeding experiments.

Another use of the theory of probable error is shown in the following example, the data for which were worked out by Mr A. B. Bruce, M.A., late Assistant Secretary of the Cambridge University Department of Agriculture, now Inspector at the Board of Agriculture and Fisheries. Mr Bruce worked out from the published figures of a number of feeding trials, the probable error of one animal in terms of live-weight increase *per diem* per 1000 lbs. live weight, the mean increase being taken in each case as 100. The results are given in the following table:

TABLE VII.

Description of animals	Place of experiment	Number of experiments	Total number of animals	Probable error on one animal as per cent. of live-weight increase
Fattening cattle ...	Cambridge	9	90	14·3
do. ...	Scotland	5	50	14·0
do. ...	America	2	40	13·7
Fattening sheep ...	Norfolk	7	100	14·3

The agreement between the probable errors in each case is extraordinarily close, and it seems fair to conclude that in feeding tests generally the probable error of one animal is 14 per cent. of the daily gain in live-weight.

Remembering that the probable error of the average of any number of observations, n, is found by dividing the probable error of one observation by the square root of the number of observations, it is possible to find the number of animals which must be employed to obtain any desired degree of precision. Thus, suppose it is desired to measure the relative feeding value of two widely different diets such as roots, chaff and linseed cake, and roots and chaff alone, where the live-weight increase may be expected to differ in a definite direction by 50 per cent., and suppose the experimenter is satisfied that odds of 30 to 1 amount to practical certainty, then odds of 30 to 1 in Table VI

correspond to a difference of 3·8 times the probable error. It will therefore be necessary to employ such a number of animals in each lot that the probable error is reduced to $50 \div 3{\cdot}8$ per cent., *i.e.* to about 13 per cent. This is very nearly the probable error of one animal. Consequently, if one animal were fed on each diet, a significant result might well be obtained. But, as more frequently happens, the experimenter desires to attempt to compare the feeding values of two rations which are approximately equal. In this case he cannot expect to get a difference in live-weight increase of more than 10 per cent. Again, taking 30 to 1 as the lowest odds which can be accepted as giving practical certainty that a difference in a given direction is significant, the number must be increased so that the probable error is reduced to $10 \div 3{\cdot}8$ per cent., *i.e.* 2·6 per cent. The number, n, required to give this precision is given by $\frac{\text{p.e. of 1}}{\sqrt{n}} = 2{\cdot}6$ or $\frac{14}{\sqrt{n}} = 2{\cdot}6$ or $6{\cdot}8n = 196$ or $n = 29$. To obtain a significant result in the ordinary run of feeding trials, it is therefore necessary to employ at least 29 animals in each lot. As this is often an inconveniently large number in the case of cattle, the desired precision is usually obtained by repeating the trial several times with smaller numbers. The point to insist upon is that when working with animals whose feeding capacity is so variable that the probable error of any one animal is 14 per cent. of the live-weight increase, the results of single trials with four or five animals are worthless.

There seems to be no way of surmounting this difficulty caused by the variation of individual animals. A very painstaking attempt carried out at Cambridge failed completely as shown by the following figures. In the spring of 1903, twelve two year old steers were purchased and treated alike for 10 months. At the end of this period four of them were picked out for experiment, the selection being based on the rate of increase of live-weight. The four picked out were so even in this respect that their live-weight increase per month for the past ten months was 38·3 lbs. $\pm$ 0·2 lbs., *i.e.* the probable error of any one of these animals was only about 0·5 per cent. of their live-weight increase. In addition to this their ages were similar, their past history identical, and their live-weights all within 10 per cent. Nevertheless, when put on to a fattening ration they at once began to diverge, and after three months' feeding their probable error was as usual 13 per cent. of their average live-weight increase. It seems impossible to take further precautions, and the conclusion is forced upon us that the

requisite precision in feeding trials can only be obtained by increase of numbers, or if that is impossible, repetition of the experiments.

The following table may be of use to experimenters who desire to make feeding trials. It is drawn up on the following assumptions:

1. That a precision of at least 3·8 times the probable error, corresponding to odds of 30 to 1 that the difference is a real one, is desirable.

2. That Mr Bruce's figure of 14 per cent. of the live-weight increase is accepted as the probable error of one animal in feeding trials.

3. That the point under investigation is whether the greater fattening power of a certain diet is significant.

TABLE VIII.

Percentage difference in live-weight increase which may be expected under conditions of experiment	Probable error of average result must be reduced to	This requires the number of animals given below, any fraction being counted as the next higher whole number
50	50/3·8	2
40	40/3·8	2
30	30/3·8	4
20	20/3·8	7
10	10/3·8	29
5	5/3·8	113

Probable Error of Field Experiments.

It would seem strange to conclude this paper without some reference to the degree of precision of field experiments, for such experiments have for many years formed the bulk of the work of the agricultural experimentalist. Indeed the present increasing interest in the work of the agricultural institutions throughout the country may be attributed to the pioneering work which has been done by means of local field experiments. By laying down such local plots and meeting farmers on them to inspect and discuss the results, the staffs of the various institutions have been brought into touch with the agricultural public, and a mutual understanding has resulted. But it cannot be denied that in many cases experiments have been commenced without proper consideration as to whether the precision of the methods adopted was capable of solving the problems proposed.

Proceeding as before, two questions arise: What is the probable error of a single field experiment, and how is it related to the size of the plot employed?

These points have been investigated by two independent methods. In the first case, an apparently uniform area of about an acre was marked out in the middle of a field of mangels, and divided into plots each of $\frac{1}{1000}$ of an acre. These were weighed separately by Messrs G. T. Spinks, B.A., of Trinity Hall, and F. R. Petherbridge, B.A., of Sidney Sussex College, to whom the authors are also indebted for the following calculations. The total number of plots weighed was 1050, but it was decided to exclude 150 of them situated at one side of the selected area, since the crop on this side was very markedly lower than on the other 900 plots. Applying the least square method to the results of the 900 plots, the probable error is found to be 12 per cent. for a single plot of $\frac{1}{1000}$ acre. Each plot was 7·26 yards along one row, the rows being two feet apart. The whole area comprising 900 plots included 36 rows, each row being divided into 25 plots. Adding together all the weights of the produce of the 25 plots in a row, 36 rows each measuring $\frac{1}{40}$ acre, the p.e. is found to be $\pm$ 4 per cent.

By rearranging the 900 plots into square plots each measuring $\frac{1}{80}$ acre and again applying the least square method to the results of these plots, the probable error of a single square plot of $\frac{1}{80}$ acre is found to be $\pm$ 5 per cent. Once more, taking long narrow plots 7·26 yards wide running across the 36 rows and each measuring $\frac{1}{25}$ acre in area the p.e. is found to be 7 per cent. This last result is very large, because of the gradual increase in crop along the rows. This systematic error was noted in the frequency curve for the weights of roots on 900 plots, Fig. 7.

The steady increase of crop across the field can be roughly allowed for in the following way. From the mean values of the yields of the 5 plots at each end the difference in yield over a range of 20 plots—*i.e.* between plots 3 and 23, the middle plots of the end groups of 5 plots which were averaged—can be found. Dividing this difference by 20 gives the correction from plot to plot, or more conveniently the correction from each plot to the central plot.

Applying the least square method to the results after correcting for the systematic error, the smoothed probable error is found to be $\pm$ 4 per cent. Such a correction can only be applied with any kind of justification where there are a large number of similarly treated plots. It would not be applicable in an ordinary field experiment.

TABLE IX.

	Yields of $\frac{1}{20}$ acre plots	Correction	Corrected yields
	lbs.	lbs.	lbs.
Mean 2640	2537	-12×25	2237
	2515	-11×25	2240
	2866	-10×25	2616
	2648	-9×25	2423
	2636	-8×25	2436
Difference between ends 500 lbs.	2581	-7×25	2406
	2814	-6×25	2664
	2944	-5×25	2819
	2748	-4×25	2648
Correction from plot to plot $\frac{500}{20}$ = 25 lbs.	2593	-3×25	2518
	2567	-2×25	2517
	2357	-1×25	2332
	2415	$+0\times25$	2415
	2424	$+1\times25$	2449
	2423	$+2\times25$	2473
	2399	$+3\times25$	2474
	2272	$+4\times25$	2372
	2374	$+5\times25$	2499
	2123	$+6\times25$	2273
	2273	$+7\times25$	2448
	2117	$+8\times25$	2317
Mean 2140	2001	$+9\times25$	2226
	2115	$+10\times25$	2365
	2246	$+11\times25$	2521
	2222	$+12\times25$	2522
	P. e. ±7 %		P. e. ±4 %

The second method of investigating the probable error of field experiments is to extract from various publications numbers of results of duplicate pairs of plots. For this purpose the authors have used the *Guide* to Experiments of the Department of Agriculture of the University of Cambridge for 1907, and the *Annual Reports* of the Norfolk Chamber of Agriculture from 1886 onwards. From these sources they have extracted the results of 400 pairs of duplicate plots including wheat, barley, oats, mangels, swedes, potatoes, seeds-hay. For each of these

pairs of plots they have calculated the mean, and the difference between each plot and the mean. This difference was then calculated as percentage of the mean. The percentages thus found range from 0 to 30 per cent. Assuming that the mean gives the correct result, which it no doubt does on the average of so large a number of observations, it is possible to plot the results in the form of a frequency curve. A fairly smooth and symmetrical curve results, Fig. 10, which indicates that the least square method may be applied to the results with some considerable degree of justification.

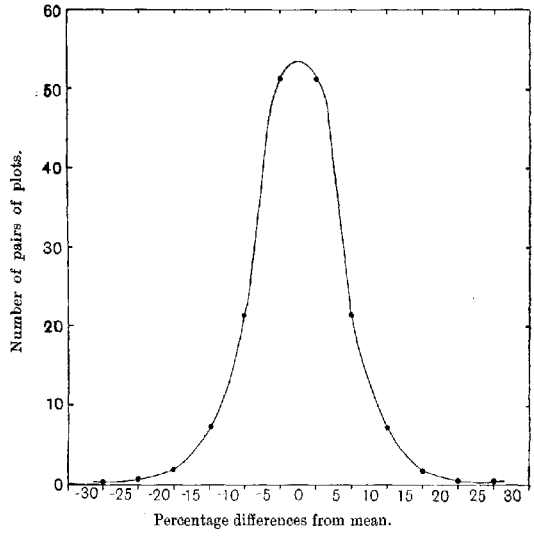

Fig. 10. Frequency curve for 400 pairs of duplicate plots.

The probable error for the whole 400 pairs of duplicates comes out 4·2 per cent. The series included plots of $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{20}$, $\frac{1}{40}$, and $\frac{1}{80}$ acre. Taking each of these separately, the probable errors are as follows:

400 pairs of plots	probable error	4·2 per cent.
45 pairs of plots each $\frac{1}{2}$ acre	„ „	3·5 per cent.
52 pairs of plots each $\frac{1}{4}$ acre	„ „	3·5 per cent.
29 pairs of plots each $\frac{1}{20}$ acre	„ „	3·9 per cent.
200 pairs of plots each $\frac{1}{40}$ acre	„ „	4·6 per cent.
75 pairs of plots each $\frac{1}{80}$ acre	„ „	3·1 per cent.

For convenience of reference the probable errors of the mangel plots already mentioned are added:

900 plots each $\frac{1}{1000}$ acre	probable error	12 per cent.
36 plots, 1 row wide, each $\frac{1}{40}$ acre	„ „	4 per cent.
25 plots, 36 rows wide, each $\frac{1}{28}$ acre	„ „	7 per cent.
do. do. do. "smoothed"	„ „	4 per cent.
25 square plots each $\frac{1}{80}$	„ „	5 per cent.

Leaving out for the present the smallest size of plot, $\frac{1}{1000}$ acre, which is obviously too small for ordinary field trials, and the smoothed result which does not compare with field plots in practice, the above table contains eight independent determinations of the probable error of field plots, all between 3 per cent. and 7 per cent. The experiments on which these results are based are known to have been carried out with every care, the whole of the produce of the plots being weighed. Many of them were on the University Farm under the direction of Professor T. H. Middleton. It seems reasonable to conclude that 5 per cent. is not too high a figure to adopt as the probable error of carefully conducted field experiments. It is noteworthy that the probable error does not depend on the size of the plot. It is not on the whole larger for the smaller plots of $\frac{1}{40}$th and $\frac{1}{80}$th acre than for the larger plots of $\frac{1}{4}$ or $\frac{1}{2}$ acre. This is quite what might be expected. By taking large plots the errors due to individual variation among the plants and to inaccuracies in weighing are, of course, reduced, but this reduction is balanced by the increase of systematic errors due to variation of soil which will obviously be likely to be greater the larger the area over which the plots extend. It is much easier to find a reasonably uniform piece of land of say $\frac{1}{4}$ acre on which ten plots of $\frac{1}{40}$th acre might be placed, than to find the two and a half acres necessary to accommodate the same number of $\frac{1}{4}$ acre plots. Nor was any distinct difference noted in going through the reports as to the error varying for different crops, except in the case of seeds-hay, where the error was on the whole above the average.

Taking 5 per cent. as the probable error of a single plot in carefully conducted field experiments, and taking 30 to 1 as the lowest odds which can be considered as amounting to practical certainty, reference to Table VI shows that the least difference between two plots which is really significant is $5 \times 3{\cdot}8$ per cent., or 19 per cent. It is 30 to 1 that a greater difference than this is not a chance difference due to normal variation of soil and other conditions, but is due to the conditions varied in the experiment.

It is obviously useless to try to measure differences less than about 20 per cent. by comparing single plots, whatever their size. If it is desired to measure such small differences, the number of plots must be increased, either by duplication several times in the same experiment, or by repetition of the experiment at several stations, or for several seasons.

The following table gives the number of plots necessary to obtain any desired precision. It is worked out on the same lines as Table VIII, for feeding experiments.

TABLE X.

Precision desired in percentage difference between yields	Probable error must be reduced to below per cent.	This requires the number of plots given below, any fraction being counted as the next higher whole number
20	20/3·8	1
15	15/3·8	2
10	10/3·8	4
8	8/3·8	6
6	6/3·8	10
4	4/3·8	23
2	2/3·8	91

For certain kinds of work very small plots of $\frac{1}{1000}$ acre or even $\frac{1}{5000}$ acre may be advisable. This is specially the case where it is desired to determine the cropping power of new varieties of cereals in the course of plant breeding work. In such cases very little seed is available and large plots are out of the question. For very small plots of about one square yard or $\frac{1}{5000}$ acre, soil variation can be reduced to a minimum by actual mixing of the soil over the very small area needed for such work. Probably therefore the probable error of one such plot can be brought down to 12 per cent., which has been shown above to be the probable error of one ordinary field plot of $\frac{1}{1000}$ acre. By duplicating such plots systematically their precision can be brought inside any desired limit. Thus for the average of nine such plots the probable error would be $\frac{12}{\sqrt{9}} = 4$ per cent., when a difference of 15 per cent. would be significant. With 16 plots the probable error would be $\frac{12}{\sqrt{16}} = 3$ per cent., when a difference of 12 per cent. would be significant. This method

has been successfully adopted by Mr E. S. Beaven, of Warminster, in his work on barley breeding. It is hoped that his results bearing on this point may appear in an early number of this *Journal*.

Summary.

Attention is drawn to the need for caution in interpreting experimental results.

Frequency curves are discussed, chiefly from the point of view of their bearing on the legitimacy of averaging results.

The method of calculating probable error is described and its meaning explained.

The application of probable error methods to questions of sampling for analysis, to field experiments and to feeding experiments, are illustrated by instances.

The probable error of one animal on a fattening ration is found to be about 14 per cent. of the live-weight increase produced, from which it is calculated that to obtain a precision of 10 per cent. in an ordinary feeding experiment 29 animals must be fed on each ration.

The probable error of field experiments is investigated by two independent methods, and found to be about 5 per cent. of the crop. This figure is shown to be independent of the size of the plot employed, provided this is $\frac{1}{80}$ acre or larger. A table is given showing the number of duplicate plots which must be employed to give any desired precision in the result.

It is also suggested that accurate results may be obtained by employing large numbers of very small plots, even as small as one square yard. This method is useful for nursery work in testing the cropping power of new varieties of cereals where very little seed is available.

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